EFSA Panel on Biological Hazards (BIOHAZ); Scientific Opinion on the public health risks related to mechanically separated meat (MSM) derived from poultry and swine

EFSA Publication

Link to article, DOI:
10.2903/j.efsa.2013.3137

Publication date:
2013

Document Version
Publisher's PDF, also known as Version of record

European Food Safety Authority

EFSA Journal 2013;11(3):3137

SCIENTIFIC OPINION

Scientific Opinion on the public health risks related to mechanically separated meat (MSM) derived from poultry and swine[1]

EFSA Panel on Biological Hazards (BIOHAZ)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The purpose of this assessment was to identify public health risks linked to mechanically separated meat (MSM) types from pork and poultry and compare them with fresh meat, minced meat and meat preparations (non-MSM); and to select, rank and suggest objective measurement methods and values for parameters to distinguish MSM types. Microbial hazards in MSM are expected to be similar to those in non-MSM, although the risk of microbial growth increases with the degree of muscle fibre degradation, thus with the separation pressure. For the distinction between the different types of MSM and non-MSM chemical, histological, molecular, textural and rheological parameters were considered as potential indicators. The analysis of available published data suggested that calcium and, if confirmed cholesterol content, was the only appropriate chemical parameters which could be used to distinguish MSM from non-MSM products. On the basis of published data, a model was developed to derive probabilities for a product to be classified as MSM based on the calcium content. Calcium content of 100 mg/100 g, as specified in the Reg. (EC) No. 2074/2005, corresponds to probability of 93.6% for a product to be classified as MSM, according to the model developed. Calcium content alone does not allow differentiation between low pressure MSM and other meat products, and other validated tests would be necessary. Histological parameters considered include microscopic detection of different tissues and their changes. The latter is a promising method for distinction of MSM types, but further validation is needed. In order to improve methods for MSM identification, specifically designed studies for the collection of data obtained by standardised methods on indicators such as calcium and cholesterol should be undertaken, while studies based on combinations of different parameters could also be useful.

KEY WORDS

Mechanically separated, recovered, deboned meat, microbial, hazards, pressure, calcium

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[3] Acknowledgement: The Panel wishes to thank the members of the Working Group on public health risks related to mechanically separated meat (MSM) derived from poultry and swine: John Sofos, Declan Bolton. Kostas Koutsoumanis, Matthias Upmann, Massimiliano Petracci and Miroslaw Michalski for the preparatory work on this scientific opinion and EFSA staff: Alessandro Broglia and Fulvio Barizzone for the support provided to this scientific opinion.

Suggested citation: EFSA Panel on Biological Hazards (BIOHAZ); Scientific Opinion on the public health risks related to mechanically separated meat (MSM) derived from poultry and swine. EFSA Journal 2013;11(3):3137. [78 pp.] doi:10.2903/j.efsa.2013.3137. Available online: www.efsa.europa.eu/efsajournal

SUMMARY

Following a request from the European Commission, the Panel on Biological Hazards (BIOHAZ) of the European Food Safety Authority (EFSA) was asked to deliver a scientific opinion on the public health risks related to mechanically separated meat (MSM) derived from poultry and swine.

Mechanically separated meat as defined in Regulation (EC) No 853/2004 is obtained by removing meat from flesh-bearing bones after boning or from poultry carcasses, using mechanical means and resulting in the loss or modification of the muscle fibre structure. Based on the current EU Regulation, low and high pressure MSM products are defined according to the alteration of bone structure and calcium content. The EU upper limit for low pressure MSM is 100 mg/100 g (1000 ppm) calcium. MSM with calcium concentration above this threshold is considered to be high pressure MSM. Different interpretations of the definition of MSM has led to low pressure MSM products being considered as meat preparations by some Member States.

Therefore EFSA was asked to issue a scientific opinion on the public health risks related to different types of MSM (high and low pressure) with a focus on low pressure MSM made with new production methods and, in particular, i) to identify the public health risks linked to the different types of MSM and compare them as well with fresh meat, minced meat and meat preparations, as defined in EU legislation; ii) to identify and rank the parameters (e.g. muscle fibre modification, calcium content, water activity) that may be used to distinguish between the different types of MSM and compare them as well with fresh meat, minced meat and meat preparations, as defined in EU legislation; iii) to establish the values for such parameters; and, iv) to propose objective methods (not subject to different interpretation) to measure such parameters.

Concerning public health risks related to MSM, the microbial hazards that may be present in MSM depend on the hygiene of processing, the levels and types of contamination present in the raw materials and their storage history, so microbial hazards in pork and poultry MSM are expected to be similar to those in fresh meat, minced meat and meat preparations. Nevertheless the risk of microbial growth increases with the degree of muscle fibre degradation and the associated release of nutrients and more uniform spreading of contamination, thus high pressure MSM may provide a more favourable substrate for bacterial growth compared with low pressure MSM.

For distinction between the different types of MSM and their comparison with non-MSM (fresh meat, minced meat and meat preparations) chemical, histological, molecular, textural and rheological parameters were considered as potential indicators.

Chemical parameters include calcium, phosphate, ash, iron, lipid (including cholesterol) and fatty acids (including those originating from bone marrow), moisture or water content, and protein (including collagen). The analysis of available data derived from published studies, albeit not specifically designed for this purpose, suggested that calcium content was the only appropriate chemical parameter that could be used to distinguish MSM from non-MSM products. Low pressure MSM contains fewer bone particles than high pressure MSM and consequently lower calcium content. Therefore calcium content alone does not allow differentiation between low pressure MSM and other meat products. The method specifically standardised for calcium determination in MSM is a titration method of the acid digested MSM using ethylene diamine tetra-acetate (EDTA), but any method providing validated results could be used.

Cholesterol content could be also useful for discrimination of MSM from non-MSM but this should be confirmed by additional data obtained by standardised methods. For other chemical parameters (protein, ash and iron) statistically significant differences were observed between MSM and non-MSM, however, the discriminatory power was very low due to overlapping data.

Histological parameters considered include microscopic detection of muscle, connective and adipose tissues, bone particles, cartilage, bone marrow and tissue from central nervous system, and their

structural changes. Among these, the microscopic examination of tissue structure changes is a promising method for distinction between MSM types and non- MSM, but further validation is needed and objective threshold values are not yet available. Among the microscopy-based methods, the detection of bone particles indicated the presence of MSM, but not all types of MSM contain bone particles. Therefore, bone particle detection may not be used alone to consistently distinguish between MSM and non-MSM. Other histological parameters related to tissue composition (muscle, connective tissue, adipose tissue, cartilage, bone marrow, central nervous tissue) do not provide clear differentiation between MSM and non-MSM.

Molecular parameters were also considered, including assays based on proteomics, metabolomics, electrophoretic techniques and immunological methods, although validation of these methods is incomplete and their cost and complexity may limit their application.

Textural and rheological properties were not considered useful to discriminate different types of MSM from fresh meat, minced meat, and meat preparations because the analysis should be carried out on products with homogeneous structure rather than on particle-reduced products such as minced meat or low pressure MSM.

A binary logistic model was developed in order to derive probability values for a product to be classified as hand deboned meat or MSM based on the calcium content. Calcium contents of 21, 39, 81.5 and 100 mg/100 g correspond to probabilities of 10%, 50%, 90% and 93.6% for a product to be classified as MSM. The distinction of low pressure MSM from non-MSM products would need to be confirmed by the addition of other validated tests for parameters such as cholesterol content and microscopic detection of muscle fibre damage.

The BIOHAZ Panel recommends that, based on changes in processing and properties of derived MSM products, the classification of raw meat recovered after deboning should be based on certain parameters of the final products, such as calcium content. New terminologies may be needed for low and high pressure MSM, because technological advances have resulted in low pressure products resembling minced meat.

It is further recommended that, in order to improve methods for MSM identification, specifically designed studies for the collection of data obtained by standardised methods on potential indicators, especially calcium and cholesterol, should be undertaken. Additional analysis in these studies could include histological examination.

Finally, it is advised that studies on differentiation of MSM from other meat products based on the analysis of combination of different parameters (chemical, physical, etc.) should also be undertaken.

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BACKGROUND AS PROVIDED BY EUROPEAN COMMISSION

In accordance with Point 1(14) of Annex I to Regulation (EC) No 853/2004[4] and Article 3 (1) (n) to Regulation (EC) No 999/2001[5], MSM is defined as follows: "Mechanically separated meat" or "MSM": means the product obtained by removing meat from flesh-bearing bones after boning or from poultry carcases, using mechanical means resulting in the loss or modification of the muscle fibre structure.

Within MSM two subtypes are identified; low pressure and high pressure MSM. Low pressure MSM is referred to in point 3 of Chapter III, Section V of Annex III to Regulation (EC) No 853/2004 as "MSM produced using techniques that do not alter the structure of the bones used in the production of MSM and the calcium content of which is not significantly higher than that of minced meat". The calcium content shall not exceed 1000 ppm of fresh product[6].

High pressure MSM is referred to in point 4 of Chapter III, Section V of Annex III to Regulation (EC) No 853/2004 as "MSM produced using techniques other than those mentioned in point 3 of Chapter III, Section V of Annex III to Regulation (EC) No 853/2004".

The reason to distinguish between the two types of MSM is that, according to the degree of reduction of the product, the vulnerability to microbial deterioration increases and, as a consequence, the risk to public health.

That is why high pressure MSM, the most reduced product, can only be used in heat treated products, while low pressure MSM, which is the less reduced MSM type, may be used in meat preparations when it complies with the microbiological criteria for minced meat.

Because of this difference in vulnerability it is necessary to compare in the opinion the requirements not only for high and low pressure MSM, but to compare them also with other reduced products like minced meat and meat preparations.

In the current discussion with the Member States there is no difference of opinion on the status and requirements for high pressure MSM, but on the status and requirements for low pressure MSM the views differ substantially. Therefore the focus of the opinion should be on the public health risks and requirements for low pressure MSM.

The most recent scientific opinion on MSM (report on mechanically separated meat health rules applicable to the production and use of mechanically separated meat) was issued by the Scientific Veterinary Committee on 16 September 1997[7]. Since then technology has evolved and for this reason an update of the scientific advice is needed in order to align the Commission's policy with current science and technology. Because of the innovative nature of the MSM industry new production methods have been developed which in particular have to be taken into account in the opinion.

Certain Member States have recently performed scientific research on MSM and the associated reports are attached to this mandate to be used as information for the required opinion.

In accordance with Article 13 of Regulation (EC) No 853/2004, the Commission shall consult EFSA on any matter that could have a significant impact on public health.

[4] Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific rules for food of animal origin (OJ L 226, 25.6.2004, p. 22).

[5] Regulation (EC) No 999/2001 of the European Parliament and of the Council of 22 May 2001 laying down rules for the prevention, control and eradication of certain transmissible spongiform encephalopathies, as amended by Regulation (EC) No 1923/2006.

[6] Point 1 of Annex IV to Regulation (EC) No 2074/2005 laying down implementing measures for certain products under Regulation (EC) No 853/2004.

[7] http://ec.europa.eu/food/fs/sc/oldcomm4/out16_en.html

TERMS OF REFERENCE AS PROVIDED BY EUROPEAN COMMISSION

EFSA is asked to issue a scientific opinion on the public health risks related to different types of MSM (high and low pressure) with a focus on low pressure MSM made with new production methods and, in particular:

1. identify the public health risks linked to the different types of MSM and compare them as well with fresh meat, minced meat and meat preparations, as defined in EU legislation[8];

2. identify and rank the parameters (e.g. muscle fibre modification, calcium content, water activity) to distinguish between these different types of MSM referred to in ToR 1 and compare them as well with fresh meat, minced meat and meat preparations, as defined in EU legislation;

3. establish the values for the parameters referred to in ToR 2;

4. propose objective methods (not subject to different interpretation) to measure the parameters referred to in ToR 2 and 3.

[8] Annex I to Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin (OJ L 139, 30.4.2004, p. 55)

ASSESSMENT

1. Introduction

In the present opinion only MSM from pork and poultry are considered, since, according to Reg. (EC) No. 999/2001, the production of MSM from ruminants originating from countries or regions with a controlled or undetermined BSE risk is currently not allowed. The present opinion does not address the detection of MSM in meat preparations, but only the identification, ranking and objective measurement of parameters that may distinguish between the different types of MSM and compare these with fresh meat, minced meat and meat preparations.

Mechanically separated meat is defined in Regulation (EC) No 853/2004 (Annex I, point 1.14) as "the product obtained by removing meat from flesh-bearing bones after boning or from poultry carcasses, using mechanical means resulting in the loss or modification of the muscle fibre structure" and specific requirements for its production are described in Annex III, Section V of the same Regulation.

The legal requirements of raw materials used in the production of different meat products (minced meat, meat preparations, and MSM) are[9]:

1. The raw material used to prepare minced meat must meet the following requirements:

(a) It must comply with the requirements for fresh meat[10];

(b) It must derive from skeletal muscle, including adherent fatty tissues;

(c) It must not derive from:

  (i) scrap cuttings and scrap trimmings (other than whole muscle cuttings);

  (ii) MSM;

  (iii) meat containing bone fragments or skin;

  (iv) meat of the head, with the exception of the masseters, the non- muscular part of the *linea alba*, the region of the carpus and the tarsus, bone scrapings and the muscles of the diaphragm (unless the serosa has been removed).

2. The following raw material may be used to prepare meat preparations:

(a) fresh meat;

(b) meat meeting the requirements of point 1;

(c) if the meat preparation is clearly not intended to be consumed without first undergoing heat treatment:

  (i) meat derived from the mincing or fragmentation of meat meeting the requirements of point 1 other than point 1(c)(i);

  (ii) MSM meeting the requirements of Chapter III, point 3(d).

3. The raw material used to produce MSM must meet the following requirements.

(a) It must comply with the requirements for fresh meat;

(b) The following material must not be used to produce MSM:

  (i) for poultry, the feet, neck skin and head;

  (ii) for other animals, the bones of the head, feet, tails, femur, tibia, fibula, humerus, radius and ulna.

[9] Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific rules for food of animal origin

[10] See Glossary

In Figure 1, Branscheid and Judas (2011) categorises meat products according to the criteria found in EU Regulation 853/2004.

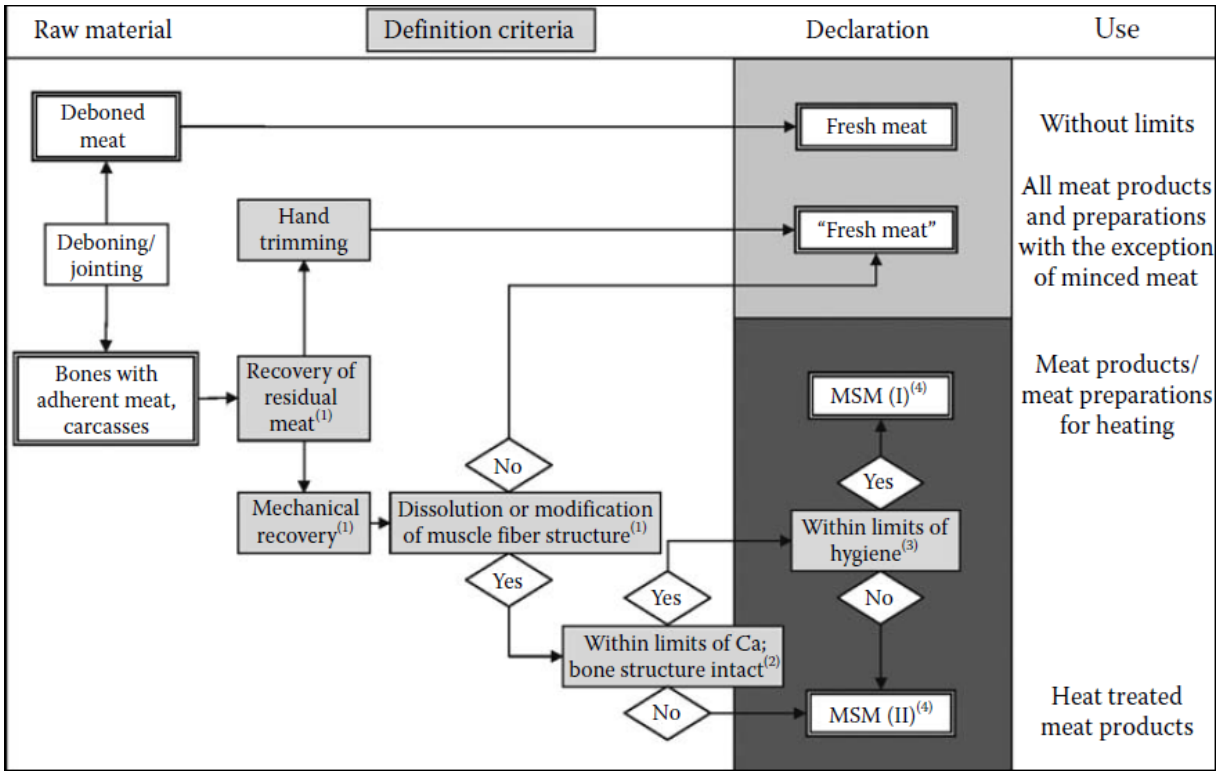

Figure 1: Definition of meat products according to criteria listed in the EU Regulation (Branscheid and Judas, 2011). (1) Regulation (EC) Nr. 853/2004, Annex III section V chapter III number 3 and 4; (2) Regulation (EC) Nr. 2074/2005, Annex IV number 1; (3) Regulation (EC) Nr. 2073/2005, Annex I, chapter 1, number 1.7 and chapter 2, number 2.1.7; (4) Directive 2001/101/EC, whereas no. 7.

In this framework, MSM is generally characterized by three properties:

- MSM is produced from meat residues that adhere to bones after deboning, and not from deboned meat;
- These meat residues are mechanically extracted;
- The extraction results in loss or modification of muscle fibre structure.

At the present time there appear to be some difficulties in the implementation of this definition of MSM in Europe. For instance, not only "flesh-bearing bones after boning or poultry carcasses" are used as raw material to produce MSM, but also deboned meat is used. This creates issues and differences among Member States. In some Member States, the product is only considered as MSM if all the elements in the above definition of MSM are complied with. In others, the derived product is considered as MSM if a technology for mechanical separation has been used, even if the raw material contains no flesh-bearing bones after boning or it is from poultry carcasses, or if there is no clear loss or modification of the muscle fibre structure.

Apart from the labelling issues, a clear definition of MSM is required to ensure food laws such as Regulation (EC) No 999/2001[11], concerning the recovery of meat from ruminant bones using mechanical methods, is understood and complied with.

Another important issue is related to the production techniques used. According to the Regulation, they alter or do not alter the structure of the bones used in the production of MSM and may affect calcium content. Based on these criteria, two MSM subtypes can be identified and are currently described as "low pressure" and "high pressure" MSM, although no clear value and/or threshold of pressure applied to the raw material is indicated by the manufacturers. The pressure used may vary with machine type and settings used, and almost all the machines used for MSM may produce both types of product by adjusting the pressure settings. Member States mostly indicate pressures below 10^4 kPa (equal to 100 bar) for the production of low pressure MSM, while pressures most often indicated for the production of high pressure MSM are above 10^4 kPa (up to $4x10^4$ kPa or more). Still these value ranges are not clear cut and there are some overlaps in pressures used between the two methods of production (EC, 2010).

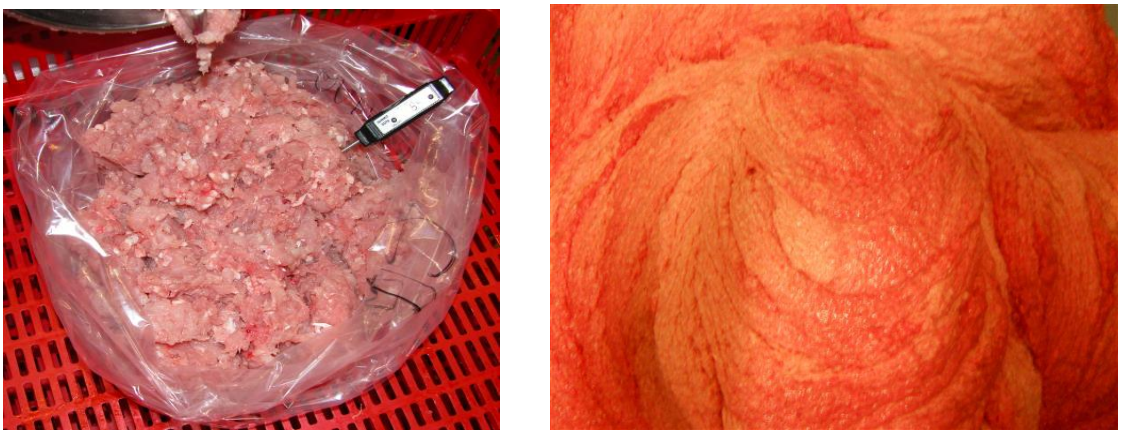

Figure 2: Low pressure (left) and high pressure (right) MSM (Petracci, 2012)

When MSM is produced using low pressure processes that do not alter the structure of the bones it is referred to as "low pressure MSM". The calcium content of low pressure MSM is often not significantly higher than that of minced meat. In particular the calcium content for MSM, as referred to in Regulation (EC) No 2074/2005 shall not exceed 100 mg/100 g (=0.1% or 1000 ppm) of fresh product as determined by a standardised international method. High pressure MSM produced using techniques other than those mentioned for low pressure MSM is used only for heat treated products because of the higher microbial contamination and potential for deterioration.

It is noteworthy that rapid and major recent technological developments in this area have resulted in the situation where some technologies for low pressure recovery of meat are able to provide a final product with characteristics close or similar to those of minced meat.

In addition to the above, some methods used to produce MSM are also used for the removal of meat from bones after heat treatment. However, only fresh meat, as defined in Annex I to Regulation (EC) No 853/2004, is considered as raw material for production of MSM. Methods for official laboratory testing of products used in order to ascertain the presence of MSM in processed meat products are not able to distinguish if the raw material was MSM or product obtained from bones after heat treatment. This results in difficulties during enforcement of official controls by some Member States (EC, 2010).

The legal hygiene requirements of raw material and of derived MSM are shown in Tables 1 and 2 below.

[11] Article 9 refers to countries with undetermined or controlled risk of BSE. If in the future some countries are considered to be at negligible risk for BSE, they will formally fall outside this case.

Table 1: Hygiene requirements of raw materials for MSM according to Regulations (EC) No 853/2004 and 2074/2005 (EC, 2010).

Raw material	Low pressure MSM	High pressure MSM
Poultry carcases	Maximum 3 days old	Maximum 3 days old
Other raw material from on-site slaughterhouse	Maximum 7 days old	Maximum 7 days old
Other raw material from other site	Maximum 5 days old	Maximum 5 days old
Mechanical separation	Immediately after de-boning	If not immediately after deboning, storage and transport at < 2°C or freezing at < -18°C of the bones (no refreezing)

Table 2: Hygiene requirements of MSM after production (EC, 2010).

	Low pressure MSM	High pressure MSM
Storage if not immediately used	Wrapped and packaged, chilling at max 2°C or frozen at an internal T of < -18°C	Wrapped and packaged, chilling at max 2°C if processed within 1 to 24h; if not, frozen within 12 h after production, reaching at an internal temp of < -18°C within 6 h. Maximal storage of frozen MSM of 3 months at < -18°C.
Use	If the food business operator has carried out analyses demonstrating that MSM is complying with the microbiological criteria for minced meat: – in meat preparations which are clearly not intended to be consumed without first undergoing heat treatment – in meat products If the MSM is not complying with microbiological criteria: only in heat-treated meat products produced in approved establishments	Only for heat-treated meat products produced in approved establishments
Calcium content	Max. 0.1% (= 100 mg/100 g or 1000 ppm) of fresh product	Not defined

2. Methods for meat recovery/deboning

2.1. Meat recovery through mechanical methods

Recovery of meat from the bones of filleted fish was the first application of mechanical flesh separation, which began in Japan in the late 1940s and increased as the amount of filleted fish produced increased. Mechanical recovery of poultry from necks, backs and other bones with attached flesh started in the late 1950s. Removal of beef and pork from irregularly shaped bones began in the 1970s (Field, 2004).

The original aim of MSM technology application was to reduce the rate of repetitive strain injury (RSI) of workers caused by short cyclic boning work in cutting rooms of meat operations. The use of a press was developed for this purpose. This technology was quite successful and was spread all over Europe and the USA within a reasonably short period (CEN, 2010).

Although mechanical meat separators have been further improved since their introduction, the mode of action of the earliest machines is still the basis of much of today's machinery.

In the beginning, primitive presses derived from other types of industries were used to separate the meat from the bones, using pressures of up to 200 bar. This yielded a fine textured meat paste suitable for use only in cooked sausages. Over the years, gradual technological improvements and pre-selection of the different types of flesh bearing bones pressed at much lower pressure (up to 20 bar) produced a coarse texture higher quality meat that could no longer be distinguished from traditional minced meat (so called 3 mm or Baader meat) (CEN, 2010).

Currently, there are three basic types of deboners on the market: 1) belt-drum system, 2) auger type, and 3) hydraulically powered presses (Barbut, 2002).

The belt-drum system (e.g. Baader and SEPAmatic systems, Figure 3) was initially developed for fish, but it was also used for poultry. In this system, the tissue is passed between a rubber belt and a micro-grooved steel drum (Barbut, 2002). Holes in the stainless steel drum range from 1 to 10 mm in diameter. Meat passes through the holes, while bones, skin and thicker layers of connective tissue remain on the outside of the drum and are ejected through a discharge chute. Pressure on the belts can be adjusted, and sometimes, pressure rollers are used to ensure an even distribution of the tissue on the belt. Following deboning, the derived mince may be refined by passing it through a strainer that removes most particles and small pieces of belly lining. Holes in strainers typically range from 1 to 2 mm in diameter. The mince can range from a coarse texture to a fine paste depending on source material, machine type and setting, and processing method (Field, 2004).

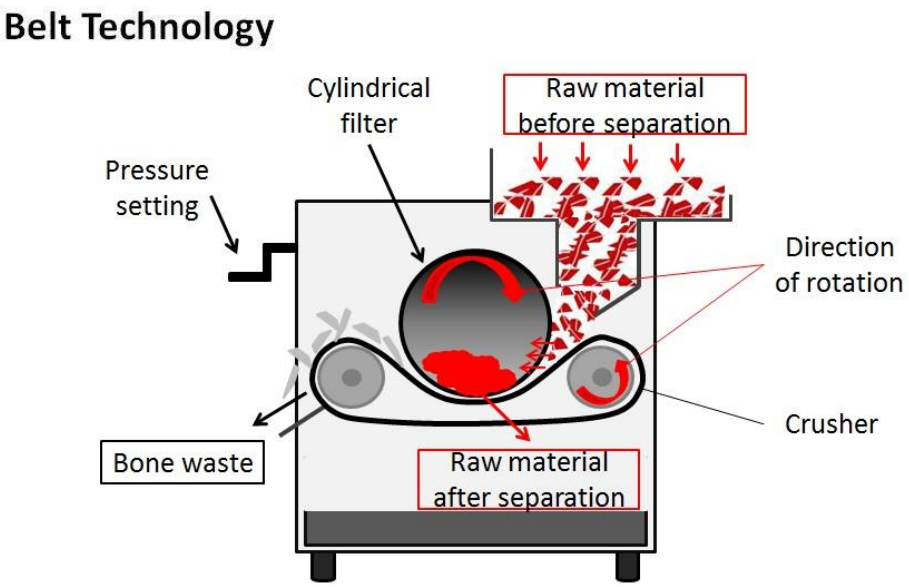

Figure 3: Scheme of belt-drum system (Source: Histalim).

The rotating auger system (e.g. AM2C, BEEHIVE, Townsend, Marel, LIMA and CFS/GEA) is used for fish, poultry and red meat. In this case, the bones and carcasses go through a bone cutter that reduces their size. The ground bone and meat mixture is introduced into a screw-driven boning head. The material is pressed (with increasing pressure), and the meat is squeezed out through the perforated steel cylinder encasing the auger. The size of the holes can be adjusted and is usually around 0.5 mm. The bone and connective tissue particles that cannot pass through the perforated cylinder are pushed forward and exit at the end of the head (Barbut, 2002).

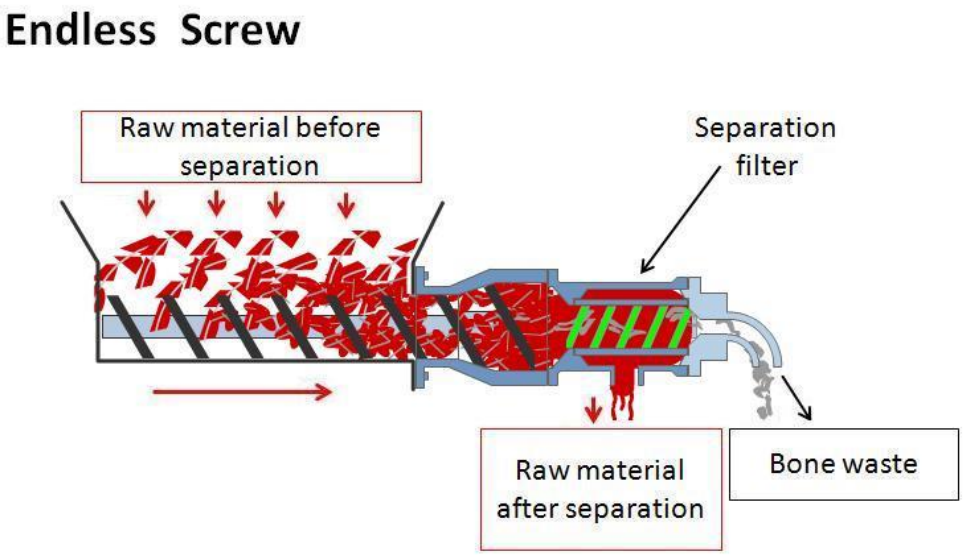

Figure 4: Scheme of endless screw technology (Source: AM2C).

The hydraulically pressed batch system (e.g. Protecon, Townsend and Marel) has been used mainly for red meat, but also for fish and poultry. The steps involved in the process are (a) presizing, (b) pressing and (c) desinewing. Presizing consists of dividing the bones into sections 10-15 mm in length. Bone sections are then pressed at high pressure in a piston-like device with holes in the walls and the pressing head. As bones are crushed and compressed, meat is pushed off the bone, through filters and away from the machine via the product outlet. Compressed bone is then ejected from the chamber and

another batch of presized bone enters. Recovered meat is transferred to a desinewing step where it passes between a belt and a drum with holes 1.0-1.3 mm in diameter. Sinews, cartilage and bone particles are removed at this step and the product is ready for use (Field, 2004). In this type of machinery, at pressures around 180 bar, meat begins to flow first, followed by fat and some connective tissue, while heavy connective tissue and compacted bones remain within the chamber (Figure 5).

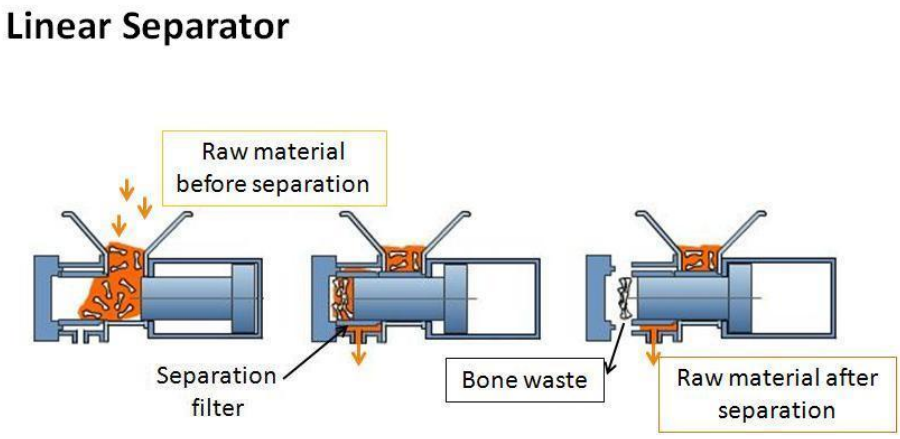

Figure 5: Scheme of linear separator (Source: AM2C).

It is a common practice that MSM production process takes place in a two-step technology, by combining press or endless screw technology followed by belt-drum separation. In the first step the meat is extracted from the crushed bones by pressure; then the belt-drum system refines the material by removing cartilages residues and thick connective tissue layers out.

Machine settings and parameters needed for MSM production include, among others, discharge plate hole diameter, drum perforation diameter, machine speed, machine tension, pressure in various modules, pressure time yield, and meat cut fed.

Meat recovered by auger type machines set at high pressure and by hydraulically powered presses falls within the definition of "mechanically separated meat (MSM)" given in Section V, Annex III of Regulation (EC) No. 853/2004 because of the high pressure used which causes bone disruption and loss or extensive modification of the muscle fibre structure. For this reason, the derived product must be used only in the manufacture of heat-treated meat products (e.g. frankfurters, nuggets, etc.). Even macroscopically, high pressure MSM is clearly distinguishable as a product with a characteristic and particularly pasty texture resulting from the loss or modification of the muscle fibre structure.

On the other hand, the belt-drum system can operate at low pressure to produce the so-called low pressure MSM or "Baader meat" or "3 mm meat" or "desinewed meat" according to different terminologies used in the meat sector. The latter can be defined as meat from which the sinews, tendons, cartilages and thicker collagen layers are removed. Pork MSM is commonly obtained from meat trimmings, while poultry MSM frequently originates from carcasses after deboning, thigh, wishbone and drumstick meat after bone removal with automated cutting equipment. Processing conditions (i.e. pressure, hole diameter, etc.) adopted to yield low pressure MSM in most cases results in less degradation of the muscle fibre structure of the meat. Thus, in some EU countries the derived product is used in the manufacture of meat preparations (i.e. meat balls, sausages, etc.) (Section V, Annex III of Regulation (EC) No. 853/2004). Still, however, according to EC requirements, low pressure MSM should be labelled as MSM and not as fresh meat or meat preparation.

It is important to note that advances and developments in equipment and processes for meat separation or recovery from bones and other structures continue. Such developments and new knowledge should be taken into account when they happen because the current knowledge may be outdated in the future.

2.2. Meat recovery through other than mechanical methods

Apart from mechanical methods, recovery of meat from bones may be accomplished by other technologies, some of which are described below (Newman, 1981).

2.2.1. Biochemical methods

Various proteolytic, collagenolytic and elastolytic enzymes have been suggested for use in meat separation from bones, but control of the process is difficult as the enzyme needs to be inactivated in the final product. This may also alter the properties of the derived product. Further, the enzymes presently available are not optimal for this use.

2.2.2. Chemical methods

Dilute acids and alkalis are effective in flesh removal, but the process leads to breakdown of proteins as well as dissolving of bones, especially the acid treatments. The resulting products are suitable only for use in the manufacture of sausages and similar formulated products.

2.2.3. Physical methods

Thermal: Cooked meat is separated from bones through pressure generated by paddles forcing it against a perforated grid. The disadvantage is that the material has been cooked and has lost its binding capacity; however, it still finds uses in the processed food industry.

Ultrasonic: This involves ultrasonic vibration of ground meat-bone homogenates in the presence of an extraction solvent. The product has the consistency of thin honey and has been successfully incorporated into frankfurter emulsions.

Cryogenic: The meat is frozen to temperatures of - 70 °C to - 110 °C and comminuted under known impact loadings. The different structural and mechanical properties of the meat and the bone result in a differential fragmentation and a selective comminution of the mixture. Electrostatic forces are then used as a method of separation.

Cutting techniques: Numerous patents have been granted for utilising fine liquid or gas jets to cut meat from bones. It is claimed that this assures the complete deboning of whole joints such as legs, whilst maintaining the meat almost intact and totally undenatured.

3. Public health risks linked to the different types of MSM

In the remit of this Opinion only public health risks deriving from biological hazards are considered. Following consultation with the CONTAM experts of EFSA concerning chemical hazards potentially present in the raw material for MSM production and/or in the final MSM product ready for consumption, no specific concerns were identified provided that legal Maximum Residue Limits and Maximum Levels (MRLs/MLs) are respected. Concerning those chemical hazards, which may be originating from MSM production, again no specific concerns were identified provided that they are controlled with appropriate measures under a validated and verifiable hazard analysis and critical control point (HACCP) plan. A risk assessment of potential chemical residues and contaminants in MSM was therefore not performed in this opinion.

3.1. Biological hazards

3.1.1. Introduction to relevant hazards for MSM as identified from a review of the scientific literature

MSM is usually heavily contaminated with microorganisms, which originate from the carcass raw material and its storage history and the processing environment, mainly as a result of poor hygienic measures (environment, handlers, and equipment). Improper holding temperatures during the production and storage phases allow growth and multiplication of contamination (Yuste et al., 2002). Although MSM products may be stored frozen and/or heat treated, several aspects of the mechanical recovery process, especially the small particle size and large surface area, the release of nutrient-rich cellular fluids due to tissue maceration, heat potentially generated during mechanical deboning, extensive handling, and cross-contamination and redistribution of contamination, may enhance bacterial growth. MSM is considered more perishable than fresh and minced meat (Viuda-Martos et al., 2012).

Few studies have examined pig and poultry MSM for the presence of pathogenic organisms so data are very limited. According to Regulation 2073/2005 (microbiological criteria for foodstuffs), under the process hygiene criteria, 5 samples must be taken from one batch per sampling session and tested for total viable counts (TVC) and *Escherichia coli*. All 5 samples must have TVC of less than 5×10^6 cfu/g and 3 samples must be less than 5×10^5 cfu/g. Similarly, all 5 samples must have an *E. coli* count of less than 5×10^3 cfu/g and 3 samples must be less than 5×10^2 cfu/g. Under the food safety criteria, MSM must also be tested for *Salmonella*. Five samples of 10g each must be taken from one batch per sampling period and *Salmonella* must be absent in all samples. However, the microbiological status of and the biological hazards in MSM are related to the bacteriological quality of the raw materials and a range of different pathogenic bacteria may be present in pig and poultry MSM. Bones are initially sterile and preventing cross-contamination is reliant on good hygiene practices (GHP) during the slaughter process and in the boning hall. Bacteria transferred to the bones or carcass parts will multiply rapidly under suitable conditions and all raw materials should be chilled quickly and maintained under chilled or freezing conditions during storage, transportation and mechanical separation. Of concern could be the potential rise in temperature during mechanical separation, which would support bacterial growth and multiplication. It is therefore important that MSM be rapidly chilled and used immediately or immediately frozen after processing.

Chilling retards but does not prevent bacterial growth. Psychrotrophic and psychrophilic organisms will grow under chilled conditions, especially in nutrient rich media such as MSM. Gomes et al. (2003), while investigating the effect of gamma radiation on refrigerated poultry MSM, reported an increase in psychrotrophic TVC from approximately 3.8 $\log_{10}$ cfu g^{-1} to 4.9 $\log_{10}$ cfu g^{-1} after 4 days, to 6.6 $\log_{10}$ cfu g^{-1} after 6 days and to 7.8 $\log_{10}$ cfu g^{-1} after 8 days storage at 2°C. An earlier study by Ostovar et al. (1971) had previously shown an increase in TVC from 3.35×10^5 cfu g^{-1} to 7.10×10^5 cfu g^{-1} on MSM derived from poultry immediately after slaughter as compared with MSM when the raw materials were stored at 3°C to 5°C for 3 to 5 days. In poultry MSM, TVC also increased from 3.25×10^5 cfu g^{-1} to 9.32×10^6 cfu g^{-1} after storage at 3°C for 12 days.

While TVC and total *Enterobacteriaceae* counts (TEC) or *E. coli* counts are good indicators/measures of process hygiene, the identification and assessment of the risk associated with specific biological hazards requires focused surveillance or studies testing MSM for specific pathogens. However, to date, very few of these studies have been completed. Bijker et al. (1987) assessed the microbiological quality of MSM in 9 pig and 6 poultry plants. Pork MSM TVC, TEC and *S. aureus* counts ranged from 5.6 to 7.7 $\log_{10}$ cfu g^{-1}, 3.3 to 5.8 $\log_{10}$ cfu g^{-1} and <2.8 to 4.6 $\log_{10}$ cfu g^{-1}, respectively. The corresponding counts in poultry MSM were 5.6 to 7.7 $\log_{10}$ cfu g^{-1}, 3.3 to 5.6 $\log_{10}$ cfu g^{-1} and 3.1 to 4.7 $\log_{10}$ cfu g^{-1}, respectively. These high levels of contamination were attributed to contaminated raw materials, inadequate process hygiene including a failure to clean and disinfect equipment and poor personal hygiene. Inadequate chilling, which facilitated bacterial growth during transportation and storage, exacerbated this situation. *S. aureus* may cause a range of infections including dermatitis, pneumonia and septicaemia. At room temperature these organisms are capable of producing several enterotoxins that, when ingested, cause a mild, usually self-limiting disease, with symptoms including vomiting with or without diarrhoea (Dinges et al., 2000). *S aureus* are prevalent in pigs and poultry (De Neeling et al., 2007; Hasman et al., 2010) but may also enter the food chain from the skin and mucosae of humans (Jay, 1997). Other strains persist in processing plants such as poultry abattoirs (Mead et al., 1989). In the Bijker study, the *S. aureus* may have originated from human, pig or poultry sources or may have been the result of cross-contamination from the processing environment.

In a New Zealand study of MSM, 145 samples collected at 3 different poultry MSM plants had *Campylobacter* contamination rates of 87%, 66% and 33% (On et al., 2011). With a reported incidence of 44.4 cases per 100,000 of the population, campylobacteriosis is the most frequently reported zoonotic illness in the EU (EFSA and ECDC, 2012). Human infections are caused principally by *C. jejuni*, common in poultry, and *C. coli*, which is found in pigs and chicken (Horrocks et al., 2009). Human infections usually result in gastroenteritis but post-infection acquired immune mediated neuropathies such as Guillian Barre Syndrome or Miller Fisher Syndrome may also occur. Recent studies have also suggested a link with inflammatory bowel diseases and irritable bowel syndrome (Haagsma et al., 2010) such as Crohn's Disease (Lamhonwah et al., 2005). Poultry are the primary source (Humphrey et al., 2007; Wingstrand et al., 2006) with, on average, 75.8% of fresh broiler carcasses being positive for *Campylobacter* in the European Union and 71.2% of broilers infected with the organism (EFSA, 2010). *Campylobacter* have been reported in poultry MSM (On et al., 2011).

In the same New Zealand study (On et al., 2011), coagulase positive *Staphylococci* were countable in 44%, 2% and 36% of processors' samples. *Campylobacter* and coagulase positive Staphylococcal counts of up to 3.7 $\log_{10}$ cfu g^{-1} and 4.06 $\log_{10}$ cfu g^{-1}, respectively were obtained. TVC and *E. coli* counts were also as high as 7.26 $\log_{10}$ cfu g^{-1} and 3.72 $\log_{10}$ cfu g^{-1}, respectively. This study demonstrated the persistence of biological hazards like *Campylobacter* through the chain from farm to slaughter, processing and ultimately to the MSM product and suggested that any hazard present in the animal may also be a hazard in MSM derived from the bones or carcass parts of that animal.

Available and comparable data on the occurrence of Extended Spectrum-Lactamase (ESBL)/AmpC-producing bacteria in poultry and poultry meat products are limited, but the occurrence appears to be moderate to high in poultry species in most European Member States. From the available monitoring data, the proportion of reported isolates that is resistant is highest for *E. coli* isolates found in broiler flocks (18%) and *Salmonella* isolates in broiler meat (11%). It is difficult to precisely estimate the quantitative contribution of ESBL-/AmpC-carrying *E. coli* from poultry to human infections (EFSA, 2012).

In a study performed by the National Veterinary Institute (NVRI) in Poland, microbiological analyses were conducted on 46 samples of mechanically deboned poultry meat (Pomykala and Michalski, 2008). Direct tests were performed according to the standards published by the International Standards Organisation (ISO) or the Polish organisation for standardisation: PN-EN ISO 6579 for detection of *Salmonella* spp., PN-EN ISO 6888-3 for detection of coagulase-positive Staphylococci, PN-ISO 4831 for detection of coliforms, PN-EN ISO for total colony count and PN-A-82055-12 for detection of spore forming anaerobe bacteria. Permitted TVC were exceeded in 3 samples (6.5%), *Salmonella* spp.

were detected in all samples tested. 27 samples were positive for spore forming anaerobic bacteria (58.7%), coliforms were isolated from 40 samples (87.0%), and in 35 samples (76%) coagulase-positive Staphylococci were also detected.

Human salmonellosis is the second most prevalent foodborne disease in Europe (EFSA and ECDC, 2012). Most cases are caused by the serovars *S.* Enteritidis and *S.* Typhimurium and manifest as gastroenteritis, but, as for *Campylobacter*, a link to human health outcomes such as reactive arthritis, inflammatory bowel disease and irritable bowel syndrome has been suggested (Haagsma et al., 2010). A *Salmonella* source attribution study concluded that both pigs and poultry products are contaminated with this organism and were important sources of *Salmonella* cases in the EU (Pires et al., 2011). As there are no interventions applied to swine or poultry bones or carcass parts that would reduce *Salmonella* contamination. It is therefore reasonable to conclude that pork and poultry MSM may also be contaminated with these bacteria as was demonstrated by Ostovar et al. (1971) who reported 11% of poultry MSM to be *Salmonella* positive.

In addition to *Salmonella*, *Yersinia enterocolitica* is prevalent in pigs and may be transferred to the carcass during slaughter. This is considered a significant risk in pork products (EFSA, 2011). *Y. enterocolitica* is the third most frequently reported cause of bacterial foodborne illness in Europe (EFSA and ECDC, 2012). Infection with this organism causes a form of gastroenteritis with abdominal pain that may mimic appendicitis and other complications include reactive arthritis. Pigs are considered to be the primary reservoir, although poultry may be secondary hosts.

Previous reports showed that *L. monocytogenes* is also a relevant hazard in MSM poultry products such as frankfurters (Ramos et al., 1998).

A similar MSM pathogen profile is described by Josefowitz (2008) who analysed 35 samples of turkey MSM meat. All samples showed higher counts than the limit of 50 cfu *E. coli* / g as a hygienic parameter according to the EU Regulation 2073/2005. In terms of health risk, the high numbers of colony forming units of coagulase-positive *Staphylococcus* and Clostridia were of particular concern. *S. aureus* has been described above. Clostridia, specifically *C. perfringens*, had been previously reported in poultry MSM (Ostovar et al., 1971). Food poisoning with the organism occurs when enterotoxigenic strains multiply in temperature abused food. Illness is usually brief and self-limiting. *C. perfringens* strains cause disease in humans and a range of animals including pigs and poultry.

3.1.2. Summary of biological hazards

There is no evidence to suggest that the microbial pathogens found in MSM are any different to those isolated from fresh meat, minced meat or meat preparations. Indeed, all pathogens found in MSM are derived from contaminated raw materials, bones and carcass parts. These, in turn, are contaminated when bacteria, primarily on the feathers and in the gastrointestinal tract of poultry or on the skin and in the gastrointestinal tract of pigs are transferred to the poultry or pig carcasses during slaughter. Cross-contamination from equipment and other environmental sources is also well documented in poultry and pig abattoirs, as well as storage and transport of the raw material to processing plants as critical point for microbial contamination and/or microbial growth. MSM, especially high pressure MSM, does however differ from fresh meat, minced meat and meat preparations in the degree of muscle fibre destruction, which tends to be more extensive. Such damage releases intracellular fluids rich in nutrients and of low acidity that supports bacterial growth (Field, 1988; Froning, 1981).

Minimising the microbial risks associated with MSM is therefore reliant on the operation of effective HACCP plan and a supporting prerequisite programme (GMP/GHP) in the abattoir and boning hall, and the efficient chilling of low pressure MSM and frozen storage of high pressure MSM. As required by Regulation the latter should also be used exclusively for cooked products.

4. Parameters to distinguish between the different types of MSM, fresh meat, minced meat and meat preparations

A clear distinction of the different types of MSM based on objective and measurable parameters of the end product is a difficult task because of the high variability of these products in their chemical and physical properties. These are largely influenced by the raw material processed and its natural variation within and between animal species, the anatomical parts to be processed, and the machinery and processing conditions. Chemical composition, muscle fibre structure, textural and rheological properties of MSM are explored here as possible parameters for MSM categorisation.

4.1. Chemical composition

The composition of mechanically recovered meat varies depending on the type of machine, anatomical location of bones, animal species, temperature, and amounts of lean meat (Field, 1988).

The chemical composition (e.g. calcium, phosphate, ash, iron, lipids, moisture and protein) of the different classes of meat has been investigated as a mean of elucidating potential chemical markers for the detection of MSM. However the results of most of these studies showed high variability depending on the kind of raw material and the technical conditions used during meat recovery. A range of values from published studies for chemical composition of MSM compared to hand deboned meat is displayed in Appendix A of this opinion.

4.1.1. Calcium

Due to the MSM production process (in particular high pressure methods), bones are crushed and an elevated amount of bone particles is to be expected in such meat. These particles contain high levels of calcium. In MSM, bone content and consequently calcium content are generally higher as compared to fresh meat (Mayer et al., 2007). Therefore, the calcium content is frequently used as one of the criteria to identify MSM, although the starting material can also affect the amount of calcium in MSM. Calcium increases during calcification processes, it varies with bone type (trabecular or compact), as well as with species, feeding or age of the slaughtered animals (Stenzel and Hildebrandt, 2006).

Calcium level and bone residues significantly increase when the extraction pressure increases (Table 3). In many countries the calcium content in the meat is regulated. In EU the maximum calcium level for the so-called low pressure MSM is 100 mg/100 g (1000 ppm). Therefore, a machine should be adjusted so as not to exceed this limit. In addition to percentage of calcium, bone particles and their size are also of great importance, because large particles might cause a gritty texture and potential dental problems. Therefore, bone particle size is regulated in places like the United States, where 90% of the bone particles cannot exceed 0.5 mm and no particle should be larger than 0.85 mm.

Other bone minerals may be used for characterisation of MSM, but they are mostly related to the calcium content, such as fluoride (Fein and Cerklewski, 2001).

Table 3: Effect of deboner head pressure (1 lb/inch^2= 6.89 kPa) on the chemical composition and yields of MSM from poultry vs. composition of hand deboned meat (Barbut, 2002).

Pressure (lb/in^2)	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Calcium (ppm)	Iron (ppm)	Palmetic[a] (%)	Yield (%)
Mechanically deboned								
40	69.82^{b}	20.65^{b}	8.13^{b}	1.05^{cd}	582^{c}	10.00^{c}	23.0^{bc}	45
75	70.37^{b}	20.76^{b}	7.88^{b}	1.04^{cd}	534^{c}	11.70^{c}	22.8^{bc}	44
120	70.28^{b}	20.10^{b}	8.47^{b}	1.12^{bc}	568^{c}	10.60^{c}	24.7^{bc}	42
150	71.05^{b}	20.68^{b}	6.78^{c}	1.23^{b}	764^{b}	17.85^{b}	27.3^{b}	82
Hand deboned								
	73.20^{c}	23.67^{c}	3.10^{d}	0.94^{d}	164^{d}	6.25^{d}	20.1^{c}	—

[a]Percent of total fatty acids.

[b–d]Means in the same column with different superscripts are significantly different ($P<0.05$).

In a study by the Polish Institute NVRI (Michalski, 2009), the traditional method for obtaining MSM (hydraulic piston) was compared to techniques that do not alter the bone structure (belt-drum separator). The average content of calcium in the MSM obtained with the latter was over three times less than that in product obtained by the traditional method.

Josefowitz (2008) showed that the drum-belt technology provided lower calcium content than the rotation auger technology. Furthermore an association was observed between the occurrence of the bone particles and the anatomical origin of the deboned material within both separated groups. The calcium content of more than 2/3 of the examined samples was lower than the limit of 1000 ppm of the EU Regulation 2074/2005 (Josefowitz, 2008).

Data from the National Nutrient Database for Standard Reference of USDA[12] shown in Figure 6 demonstrate the calcium content of high pressure MSM and hand deboned (HD) meat from poultry products.

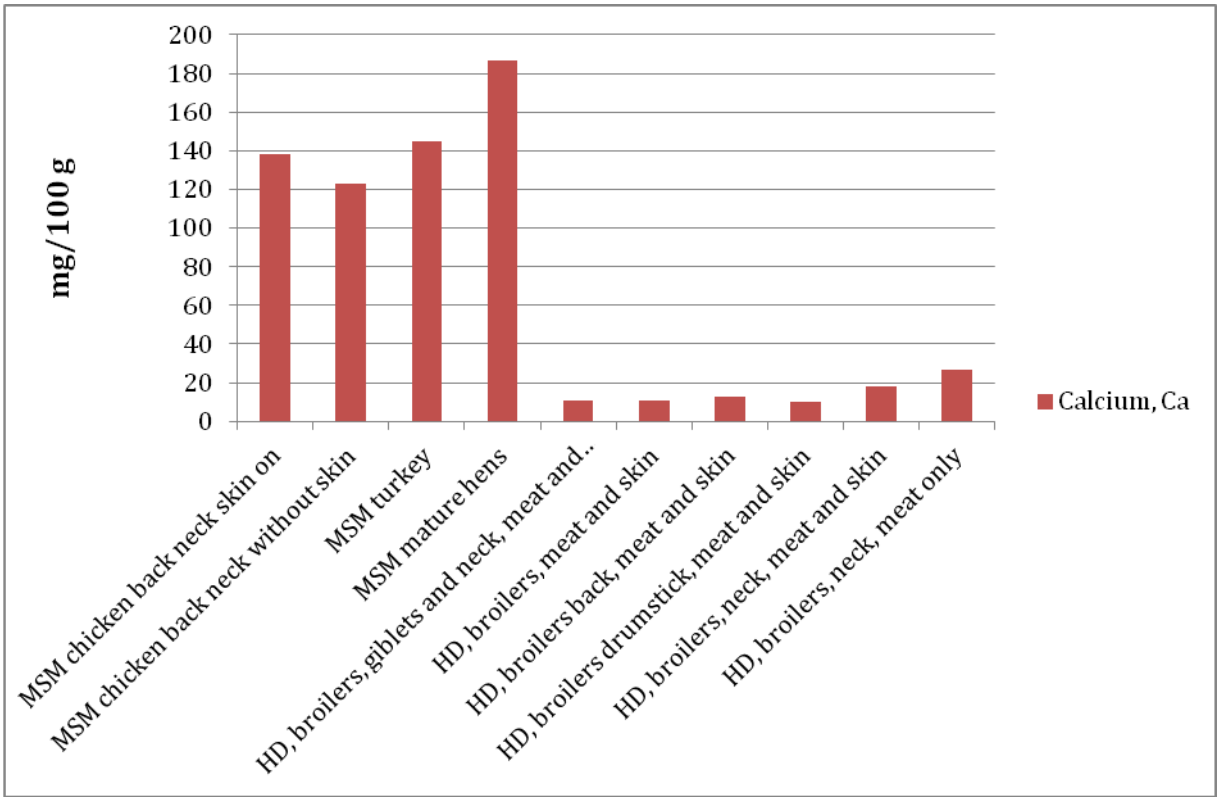

Figure 6: Calcium content in MSM and hand deboned (HD) poultry meat (data from USDA)[12]

The results of a series of studies have shown that MSM has a more elevated calcium level than hand boned meat (Ang and Hamm, 1982; Calhoun et al., 1999; Demos and Mandigo, 1995; Field and Riley, 1972; Field and Riley, 1974; Mayer et al., 2007). The chemical composition of MSM is inherently variable due to the natural variation within and between animal species, feeding regimes, age of the slaughtered animals, cuts of meat, bone type, previous treatments of bones (trimming, freezing, and so on), and the machine type and operating conditions used in the recovery process (Day and Brown, 2001; Stenzel and Hildebrandt, 2006; Viuda-Martos et al., 2012). The lowest calcium content detected by chemical analysis was in samples of deboned chicken carcass meat (0.06%), slightly higher in deboned meat samples of the back (0.19%) and neck (0.21%), and the highest in wing samples (0.29) (Botka-Petrak et al., 2011). MSM from whole carcasses of spent layers had higher calcium content than MSM from chicken backs and necks, and turkey backs (Field, 1999; Grunden et al., 1972). Much

[12] Data from http://ndb.nal.usda.gov/ndb/search/list

higher calcium contents were found in MSMs from different cuts (chicken neck, with and without skin, and back) than in manually deboned meats from the same cuts (Ang and Hamm, 1982).

During mechanical deboning of meat, it is inevitable that some bone particles pass into the MSM. These particles contain high levels of calcium. In MSM, bone content and thus also calcium content are elevated compared to fresh meat (Mayer et al., 2007) . A high bone content means that the pressure used in the deboning process was too high or that the meat to bone ratio was too low (Beraquet, 2000). Poor equipment assembly or adjustment could also lead to obtaining unacceptable particle size, affecting the quality of products that use the MSM, although, in general, presence of bones is not a problem (Newman, 1981). Calcium may also come from calcium phosphate in bone fluids which can be expressed when the bones are placed under pressure (Crosland et al., 1995).

Calcium represents 37% of the ash content of bones but both ash and calcium levels increase during calcification processes, so different conversion factors would be necessary to estimate the content of bones through calcium content or ash (Blincoe et al., 1973; Campo and Tourtellotte, 1967; Field et al., 1974).

Calcium content could be an indicator of the amount of bone in MSM. The determination of bone (or calcium) content in MSM is a form of controlling the yield of mechanical separation processes.

4.1.2. Phosphate

The effect of increased phosphorus content in MSM on human health is a controversial issue. The phosphorus content of MSM is dependent on animal species, age of the slaughtered animals, cuts of meat, bone type (cartilage, necks, wings, bones, back), previous treatment of the bones (trimming, freezing, etc.), and the machine type and operating conditions used in the recovery process (Froning, 1981; Michalski, 2009).

Phosphorus content is not considered to be a food safety or health issue. Although subject to quantitative limits in the finished meat products, there are no specified limits for MSM. MSM is generally used as a raw material (in an amount of from several to several dozen percent for the production of different meat products (homogenized sausages, meat pie, offal products) and is often the cause of increased phosphorus content in the final product offered to consumers (Michalski, 2006; Nurmi and Ring, 1999).

In a study performed by the National Veterinary Institute (NVRI) in Poland, 40 samples of poultry MSM (10 samples from goose, 29 from chicken and 1 from duck) from different factories were analyzed. Differences in phosphorus content among samples were observed (Table 4), ranging from 0.08% to 0.34% of MSM (Michalski, 2006). Contents of P in poultry meats are in the range 0.115-0.158% (USDA 1979). For pork, the phosphorus content is in the range of 490-2080 mg/kg (0.05-0.21%)[13].

Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives (OJ L 354, 31.12.2008, p. 16) gives limit values for the maximum acceptable level of phosphorus (phosphoric acid + phosphates + di-, tri- and polyphosphates) in meat and meat products. The limit is 5000 mg/kg (0.5%, expressed as P_2O_5, equivalent to 0.22% of P).

[13] Nutritional Table: http://www.dobradieta.pl/tabele.php

Table 4: Content (%) of total phosphorus in different types of poultry MSM (Michalski, 2006, 2007).

	MSM from chicken		MSM from turkey		MSM from goose	
	as P_2O_5	P	as P_2O_5	P	as P_2O_5	P
Min	0.266	0.116	0.291	0.127	0.153	0.069
Max	0.685	0.299	0.577	0.252	0.218	0.095
Mean	0.368	0.163	0.427	0.187	0.183	0.080
sd	0.076	0.037	0.078	0.034	0.019	0.008
n	158		31		10	

4.1.3. Ash

Ash content can be used for MSM detection because calcium is a relatively constant fraction of bone ash (37%) (Branscheid and Judas, 2011). Ash analysis is far less laborious and, thus, is suited for less well equipped laboratories as an alternative to calcium analysis. The correlations of bone, ash, and calcium contents were analyzed by Field (Field, 2000), who proposed respective conversion factors. These factors are also suited to approximately convert bone contents that were determined directly as ash or calcium contents. For exact results, it is necessary to determine specific conversion factors adapted to the specific material.

4.1.4. Iron

It has been extensively demonstrated that high pressure (1000 kPa) almost doubled MSM production yield, but also increased the iron content of MSM by about 70% in poultry meat (Barbut, 2002). Most of the increase in iron content has been reported to be from haemoglobin (Froning, 1981). The heme content can vary considerably depending on the bone-to-meat ratio, deboner settings, skin content and the age of the animal. The high aeration rates during the process (i.e., exposure of large surface area to air) result in converting most of the myoglobin into oxymyoglobin. The oxymyoglobin on the surface is often oxidized to metamyoglobin, thereby giving the product a brown colour. Lower temperatures can help reduce heme oxidation (Froning, 1981).

4.1.5. Lipid, fatty acids and cholesterol

Mechanical deboning of meat affects the lipid composition of the resulting meat, which normally has higher lipid content than manually deboned meats. These extra lipids may originate from subcutaneous fat, the skin or abdominal fat (depending on the animal species and method used) but mainly come from bone marrow and bone tissue (Trindade et al., 2004). The fat present in MSM is rich in polyunsaturated fatty acids due to the presence of phospholipids from the fraction of bone and accompanying spinal marrow (Viuda-Martos et al., 2012).

The process of mechanical deboning makes the meat highly susceptible to lipid oxidation due to the extensive cellular disruption and release of enzymes. In addition, exposure of a large surface area to air, during the process, and the extraction of heme and lipids from bone marrow can also make the meat more susceptible to rancidity (Froning, 1981; Field, 1988). The rate of lipid oxidation is also influenced by the pressure exerted on the meat during the deboning process. Higher pressure results in higher yield, but also increases the proportion of some of the unsaturated fatty acids.

Cholesterol content of mechanically separated pork and poultry meat is usually higher than that of hand-boned meat. This increase is essentially associated with increase in fat and marrow. Especially in poultry, skin-on cuts were mechanically-separated and this implies an increase in fat and cholesterol content because most of this is associated with skin. Moreover bone marrow released from the bones induced by mechanical deboning causes increased cholesterol content (Field, 2004; Froning and McKee, 2001; Viuda-Martos et al., 2012).

Varying amounts of fatty acids are also contained in bone marrow, ranging between 7% to 48%, depending on the animal species and even on the type of bone, since the marrow from leg bones of adult animals can contain up to 90–95% fat (Field et al., 1980). The reason that MSM has been regarded as having a potential health risk is the high concentration of various polyunsaturated fatty acids (PUFA), which may have adverse physiological effects via their (per)oxidation products (Püssa et al., 2008).

4.1.6. Moisture / water content

As in regular meat, moisture content of MSM fluctuates with lipid content, which varies considerably depending on the material being deboned (Froning and McKee, 2002; Field, 2004; Viuda-Martos et al., 2012). As a consequence, MSM contains lower available moisture than hand-deboned meat because of the higher lipid content. However, water activity is in a range allowing growth of all microorganisms in all types of such products, if unfrozen.

4.1.7. Protein (including connective tissue)

As with lipids and moisture, protein content of MSM varies depending on the material being deboned (Froning and McKee, 2002; Field, 2004; Viuda-Martos et al., 2012). Usually, however, protein content is higher in manually separated meat than in MSM because raw materials used for mechanical deboning are richer in lipids (e.g. skin, subcutaneous fat, etc.). The protein quality of MSM has received considerable emphasis; however, it seems that differences are mainly related to the relative presence of collagen instead of technological issues (Froning and McKee, 2002). Collagen exists in several forms and constitutes an integral part of meat, fat depots, tendons, cartilages, and bones. It is well-known that collagen has an inferior nutritional and technological quality compared to myofibrillar and sarcoplasmatic proteins (Viuda-Martos et al., 2012). Even if raw materials used for mechanical deboning are usually very rich in connective tissues, its content may not be different from hand-deboned meat because its high tensile strength may partially prevent its extrusion with the meat (Field, 2004).

4.2. Muscle fibre modification

EC regulation 853/2004 establishes the loss or modification of muscle fibre structure in meat as criteria for differentiation of MSM. The impact of mechanical forces on structural properties of muscle foods was studied in MSM production processes but also in connection with tumbling or tenderization of meat. In general tissues react differently when exposed to friction, bending, torsion, expansion, tear and shear forces, and the resulting changes on cellular scale may be major or minor or simultaneously occurring with transitional states.

In this respect, the changes occurring during the conversion of muscle tissue to meat are also relevant. Prior to meat cutting and deboning, the action of calcium (Liu et al., 1995) and endogenous proteolytic enzymes (Sentandreu et al., 2002) during conditioning and aging cause softening of the myofibrillar structure. As shown by Gann and Merkel (1978) using electron micrographs, myofibrils in beef were fragmented in the Z-disc-I-band junction of the sarcomere within 48 hours post mortem, with or without a limited degradation of the Z-disc itself depending on the muscle fibre type. Additionally, a disintegration of the intramuscular connective tissue was observed under light and in scanning electron microscopy of chicken semitendinosus muscle (Liu et al., 1994, 1995). Beginning with small gaps visible at endomysium junctions as early as 5 h post mortem, the endomysium and perimysium disintegrate into several thin sheets within 12 h post mortem. These processes were also present in pork and beef (Nishimura et al., 1995; Nishimura et al., 2008), although the time required for their onset was much longer (8 and 14 days post mortem, respectively). Both processes - the rearrangement of collagen fibrils and fibres as well as softening of the myofibrillar structure - result in a reduced resistance to mechanical forces with perpetuity of aging. Thus, the observable changes in muscle fibre structure of MSM will depend also on the time between slaughter and separation procedure, i.e. the duration of the aging period. According to Reg. (EC) No. 853/2004 (Annex III Section V Chapter III No. 3. and 4.) raw material intended for MSM production may be up to seven days old (poultry up to

three days) before deboning. This period may be prolonged by a subsequent chilled or frozen storage period of the flesh-bearing bones in case of usage for heat-treated meat products. Unfortunately, data concerning this aspect are lacking.

A characteristic of poultry MSM, is the heavy fragmentation of the myofibrils as well as breaks in the Z-lines and distortion of the sarcomeres, as reported by Barbut (2002). It is not clear if these signs differ significantly from those seen in aged meat (see above). After applying mechanical forces to pork during tumbling, several authors observed a disruption of the endomysium and sarcolemma, resulting in muscle fibre destruction (Dolata et al., 2005; Katsaras and Budras, 1993; Theno et al., 1978). Additional criteria were used by Cassidy et al. (1978), who evaluated the muscle cell integrity by means of four characteristics: i) clarity of striations, ii) cell membrane disruptions, iii) clarity of nuclei, and iv) disorganization of nuclei. These criteria were also suggested by Hildebrandt (2007) for the classification of MSM and non-MSM. Several studies concerning the distinction of separation technologies on the basis of muscle structure damages are based on these criteria (Branscheid et al., 2011; Branscheid et al., 2012; Groves, 2011; Henckel et al., 2011; Sifre et al., 2009). Additionally, other structural aspects such as dispersed protein and connective tissue content were used by Groves (2011) in combination with the above mentioned changes of muscle fibre integrity.

4.3. Textural and rheological properties

Rheological properties of meat include e. g. textural properties, emulsifying capacity, and thermal and electrical conductivity. These properties are relevant for manufacture-processed products (i.e. coarse ground sausages, frankfurters, restructured cooked ham) and, in general, for cooked products and/or for products with homogeneous structure. The evaluation of rheological properties in ground meats like different types of MSM is therefore not much applicable and/or scantly informative.

Mechanically separated meats are largely used to manufacture further processed products, thus the effects of the mechanical separation process on protein content and functionality and fat level have been found to influence textural and rheological properties (Froning and McKee, 2001).

Mechanically separated meats are relatively low in protein both in quantity and quality (i.e. more collagen and less myofibrillar proteins than minced meat). These can negatively influence overall protein functionality by decreasing ability to retain water during processing and storage, to emulsify lipids and to form a stable gel during cooking. However, there is a large variability in these properties in relation to sources and harvesting technologies. For example, it has been observed in poultry that a higher content of skin tissue decreases emulsion stability and capacity, which are largely related to the higher fat and collagen content contributed by skin (Froning and McKee, 2001; Viuda-Martos et al., 2012). However, Schnell et al. (1973) reported that the higher skin content increased organoleptic tenderness of frankfurters. Mast et al. (1982) evaluated some rheological properties of frankfurters manufactured by mechanically separated meat deboned by using different technologies. It was observed that differences existed in the emulsifying capacity and stability, however all meat types were successfully used in the preparation of acceptable frankfurters. Chia et al. (1999) studied possible changes in the quality of chicken sticks formulated with mechanical separated chicken meat (0-50%) mixed with hand deboned chicken breast meat. The protein content and hardness of the sticks decreased as the proportion of mechanically separated meat increased; however, the fat content and the losses due to cooking increased. When between 30 and 50% of mechanically separated meat was used, the general acceptability of the products improved, although the product was considered softer than the control. Chinprahast et al. (1997) compared the quality of nuggets prepared solely from intact muscle meat with nuggets prepared with a combination of mechanically separated meat and chicken breast meat. The best results were obtained with a 40:60 combination of mechanically separated meat and breast meat, which showed no significant differences from the 100% breast nuggets. In the product with 100% mechanically separated meat, the gel strength and adhesiveness decreased significantly.

Calhoun et al. (1999) compared ground pork patties manufactured with different ratios of finely textured trim harvested by an advanced meat recovery system. The incorporation of finely textured trim decreased hardness, chewiness and cohesiveness of ground pork patties. Finally, Petracci et al. (2012) found that poultry and pork meat patties produced by low-pressure recovery systems had higher hardness and gumminess as well as lower cohesiveness and springiness values if compared with the same products made by meat trimmed by hand and minced by conventional mincers.

As for electrical conductivity, this property is related to chemical composition of the meat (lipid, protein, collagen and minerals), therefore it depends solely on raw meat composition and does not allow discrimination between low- and high-pressure MSM.

In conclusion, it can be argued that textural and rheological properties of finished meat products are affected by use of mechanically separated meat, however their evaluation is not very useful to discriminate different types of MSM from fresh meat, minced meat, and meat preparations because the analysis should be carried out on products with homogeneous structure rather than on particle-reduced products like minced meat or low pressure MSM.

5. Methods for MSM parameter measurements

Detection methods for MSM take advantage of the changes in the product caused by the pressure used to separate meat residues from bones. This causes abrasion of bone particles, extrusion of soft tissues (bone marrow, connective tissue), and modification of muscle structure. Modifications of muscle structure are more difficult to determine objectively, compared to the various detection methods available for bone related changes. Therefore, for many years, the scientific efforts focused on the calcium content/bone fraction as a key criterion for MSM (Branscheid and Judas, 2011). Only recently, also histomorphological and molecular methods to detect modified muscle structure, and use of specific biomarkers are discussed more intensely (Skarpeid et al., 2001a; Surowiec et al., 2011a; Surowiec et al., 2011b).

5.1. Chemical methods

Chemical methods for detection of minerals in MSM may be considered as indirect methods for bone detection to be used as a parameter to distinguish different MSM types. These methods do not identify bone as a physical substrate, instead, they analyze for chemical components of bone (ash, calcium) or of collateral tissue (bone marrow, spinal cord) (Branscheid and Judas, 2011). Analytically, most important are not only mineralized components of bone but also bone marrow and its characteristic components (minerals, proteins, nucleotides). Tissue of the central nervous system (CNS), in particular from the spinal cord, can be used as an indicator of MSM from the spine.

The levels of minerals for most foods are commonly determined by methods of AOAC International[14]. Calcium, iron, magnesium, phosphorus, sodium, potassium, zinc, copper, and manganese are usually determined by inductively coupled plasma emission spectrophotometry (AOAC International method 984.27) or, except for phosphorus, by atomic absorption (AOAC International method 985.35), with phosphorus determined calorimetrically by AOAC International methods 2.019, 2.095 and 7.098. The determination of calcium in a food matrix can be performed by using atomic absorption spectrophotometric and potassium permanganate titration methods (titrimetric method). The method specifically standardised for calcium determination in MSM is AOAC International method 983.19. This is a simple titration method of the acid digested MSM with EDTA (ethylene diamine tetra-acetate). It is also usual for calcium to be determined by atomic absorption spectroscopy (AAS). Any method can be used provided that it gives validated results.

Chemical methods are used for the isolation of bone particles in meat. The standard laboratory technique used in the U.S. for bone particle determination takes 13 or more hours and relies on enzymatic digestion using papain followed by separation with carbon tetrachloride, acetone and ether. More recent techniques aim to increase the accuracy, to shorten the time required, and to avoid use of noxious reagents (McNitt et al., 2004).

Histochemical methods for detection of mineralized components are also available: at routine level two histochemical staining methods are applicable, namely staining with silver nitrate, which exclusively attaches to mineralized bone particles, and with Alizarin Red, a specific dye for calcium salts. Both allow the application of automated image analysis (Branscheid and Judas, 2011)

A combination of morphological and chemical isolation of bone particles may also be used. Bone particles can be isolated by staining or by chemical digestion of muscle tissue. The first has to be followed by manual, macroscopically controlled separation of particles, whereas chemical digestion of soft tissues allows segregation of bone particles. In either case, recovered particles have to be weighed, or the segregated particles can be reduced to ashes and quantified. Without any sample processing, bone particles may also be detected by radiologic methods (Branscheid and Judas, 2011).

For other components of MSM that may be used for MSM classification, such as cholesterol, various methods (enzymatic, colorimetric, gas-chromatography and high performance liquid chromatography)

[14] Association of Analytical Communities, http://www.aoac.org/about/aoac.htm

have been developed for determination in meats. However, colorimetric and enzymatic methods need strict control of the analytical conditions to give accurate results, so chromatographic techniques are preferred (Petracci and Baeza, 2011).

5.2. Microscopy-based methods

5.2.1. Detection of differences in tissue composition

5.2.1.1. Determination of tissue quantities

An approach to distinguish between MSM and non-MSM as well as between different kinds of MSM is the detection of typical tissue components by means of histological examination. For this purpose, thin sections of the samples are prepared, stained and inspected microscopically. The histological methods used depend on the target tissues and the accuracy required. Generally, the procedure may provide:

- Qualitative results, assessing only the presence (or absence) of distinct tissues in the sample. If the structures to detect exhibit colour differences due to staining, this process may be automated (Stenzel and Hildebrandt, 2006).

- Semi-quantitative results provide, additionally, a rough estimation of a distinct tissue's proportion in the samples. Results are indicated in frequency classes as cited from Tremlova et al. (2006): "sporadic occurrence", "negligible amount", "moderate amount", "medium amount", "considerable amount" and "prevailing".

- Quantitative results of the amount of a distinct tissue may be attained either by counting the number of tissue particles found in the histological slide (thus in a defined tissue quantity) or by use of the point-counting method in which the tissues in a specimen are evaluated stepwise in a high number of points at preset distances, resulting in a statistically based determination of the target tissue's fraction. Additionally, planimetry of digitalized slides is an increasingly used quantitative evaluation method, which provides test results on surface areas or volumes of tissue particles using computational image analysis procedures. Counting and planimetric evaluation may be automated using image analysis systems if the colour of the tissue or structure to be determined is distinct from those of other tissue compounds in the sample.

5.2.1.2. Target meat main tissues

Several authors compared the tissue composition of MSM recovered (muscle, connective, adipose tissue) with different separation techniques from different animal species and from different parts of the slaughtered animals (i.e. different meat bearing bones). In general, muscle tissue dominated in all MSM samples. However, tissue quantities showed considerable variation depending on the previously mentioned parameters.

Performing an interspecies comparison, Koolmees et al. (1986) detected muscle tissue quantities in pork MSM between 50 and 75% and in chicken MSM between 40 and 60%. Also Tremlova et al. (2006) detected more muscle tissue in MSM from pork bones ("considerable amount") than in MSM from poultry bones ("medium amount"), both produced with a press-type separator. The connective tissue accounted for 20 to 40% in pork MSM and 35 to 50% in poultry MSM (Koolmees et al., 1986), which corresponds largely to the "medium amount" of Tremlova et al. (2006).

However, recovery results are highly influenced by the bone fraction separated. Koolmees et al. (1986) found the highest amounts of muscle tissue in MSM from back, rib and shoulder bones of pigs (above 70%) as well as in MSM from poultry carcasses (above 50%). In contrast, pork heads MSM contained low muscle tissue quantities (17%) and very high connective tissue (77%) contents. Similarly, in the study of Bijker et al. (1983) pork MSM produced by a discontinuous pressure system contained mean volume percentages of muscle tissue of 48 to 86% and of connective tissue from 12 to

48% depending on carcass parts (ribs, backs, legs, shoulders and mixtures thereof). With poultry, Botka-Petrak et al. (2011) detected an increasing content of connective and adipose tissue in the order: whole carcasses < backs = necks < wings.

Concerning the separation technology, Bijker et al. (1983) assumed an increasing MSM quality (i.e. increase of muscle tissue, decrease of connective tissue) with decreasing demeating efficiency of pork bones. According to Henckel et al. (2004), the connective and adipose tissue of mechanically separated chicken meat was significantly increased (≥ 25%) in comparison with hand deboned and with minced meat (≤ 20%), although the quantity present was highly dependent on the separated bone part itself. Additionally, chicken MSM produced by continuous auger-sieve separators contained more connective and adipose tissue as that produced by hollow belt separators though the differences were small (3 to 6%). This is in agreement with results of Tremlova et al. (2006), who were not able to detect differences in the composition of auger-sieve separated poultry bones and hollow-drum/belt treated poultry trimmings by estimation (both "prevailing" muscle tissue and "moderate amount" of collagenous tissue). However, differences occurred between press-type and auger-sieve technologies when poultry bones were separated: The press-type technology produced poultry MSM with higher collagenous and lesser muscle tissue (both "medium amount") than the auger-sieve machine ("prevailing" muscle tissue, "moderate" collagenous tissue) (Tremlova et al., 2006).

Overall, differences in meat tissue composition are varying and overlapping. Quantitative detection of meat main tissues does not provide an unambiguous result for the differentiation of the separation technology nor for the pressure used.

5.2.1.3. Bone particles

Literature concerning bone particles in MSM is extensive, comprising the study of amounts, structures and sizes of bone particles since those are frequently taken as indicators for either MSM quality or for the use of MSM in processed products.

Branscheid and Judas (2011) revised the direct methods for calcium and bone detection. Among these, morphological detection by microscopy of bone and collateral tissue types can be considered. A number of cytological characteristics can be used to identify MSM: osteocytes, collagen fibres of the *tela ossea*, components of bone marrow, cartilage tissue, or firm connective tissue.

Several factors influence the occurrence of bone particles in MSM. One main factor determining the amount and size of bone particles in MSM is the separation technology used (chapter 4.1.1.). In high pressure deboned pork MSM Bijker et al. (1983) found mean volume percentages of bone ranging between 0.4 and 1.9%, being rather similar to the data given by Koolmees et al. (1986). According to the latter authors, pork MSM contained 1 to 2% bone, which was less than the content in poultry MSM (2 to 4%). In contrast, Branscheid et al. (2012) recently detected only very low bone particle quantities in poultry separates (max. 0.2%). Surprisingly, the highest content in this study was detected in hand deboned turkey lower legs. Linke and Thumser (1964) had already described, that bone particles occur sporadically also in hand deboned meat and reported volume percentages in manually separated pork of up to 0.8%. Hildebrandt and Josefowitz (2007) and Stenzel and Hildebrandt (2006) highlighted that the sporadic occurrence of bone is technologically unavoidable implying that up to 1 particle per microscopic slide is tolerable. This grossly corresponds to a maximum of 0.2 bone particles per 1 cm^2. Consequently, several authors suggested threshold levels for bone particles in meat products. According to Bijker et al. (1985) no or low amounts of MSM have been used when up to 30 bone particles occur in 8 sections while the use of MSM can be considered as certain above 60 bone particles; the interval between 30 and 60 particles is regarded as evidence that MSM might have been used in a product. This approach was later validated by Schulte-Sutrum and Horn (2003) who adapted the suggested numbers to a threshold per section with a minimum of 10 sections examined: "less than 1 bone particle", "up to an average 1.5 bone particles" and "more than 1.5 bone particles". These values are from the current German food control authorities' judging base applied to the use of MSM in meat products.

European Union MSM regulations set the unaltered (not destructed) bone structure during production as a prerequisite for the use of MSM in meat preparations (Annex III Sect. V Ch. III No 3 (d) of Reg. (EC) No. 853/2004), otherwise MSM use is limited to heat treated products. The microscopical detection of bone particles may potentially serve as an indicator for distinguishing low pressure MSM (for use in preparations) and high pressure MSM (only to be used in heat treated products), and EC regulation 2074/2005 introduced a threshold for calcium for this purpose (1000 ppm). Although the presence of comminuted bone is the underlying reason for an increased calcium level according to Branscheid et al. (2009), the correlation between bone particles and calcium content may be also influenced by the type of bone, the breed, the age, the feed as well as the physiological state of the animal from which the bone originates (Bijker et al., 1985; Branscheid et al., 2009; Branscheid et al., 2012). However, Stenzel and Hildebrandt (2006) mentioned a good agreement between both parameters. The correlation coefficient determined by Bijker et al. (1983) was r = 0.81; a similar correlation coefficient (r = 0.78) was reported by Tremlova et al. (2006).

Concerning the bone content in products from different separation technologies, Koolmees et al. (1986) observed a tendency of press-type machines to produce lower bone contents than auger-type separators in chicken MSM production. This observation was confirmed by Josefowitz (2008) for separated turkey bones. However, in press-type pork MSM particle sizes and amounts seem directly correlated with the pressure administered (Nitsch, 2005), thus probably being a question of the machine's settings. Furthermore, Josefowitz (2008) and Nitsch (2005) presumed an interrelation between the occurrence of bone particles and the anatomical origin of the deboned material.

In addition to the quantity of bone particles, the particle size is also of great importance. The size of bone particles is determined primarily by the deboning machine, operation, and the size of filter used. Koolmees et al. (1986) found that between 84.8 and 97.5% of bone particles of MSMs obtained through different deboning machines were smaller than 1.0 mm. The bone particles of MSM are totally solubilised in HCl solutions at concentrations similar to those found in the stomach and hence the author concluded that mechanically deboned red meat, poultry and fish contained bone fragments which were not hazardous to consumers (Field, 1988), however particle sizes larger than 1,5 mm^2 are sensorially unacceptable according to Nitsch (2005).

More than 90% of the bone particles in pressure-piston separated pork were smaller than 1 mm^2, although even particles larger than 3 mm^2 were detected (Bijker et al., 1985; Bijker et al., 1983). In the study of Froning (1981), mechanically deboned chicken back and neck meat contained bone particles from 80µm to 1.5 mm in diameter with an average width of 0.2 mm and an average length of 0.4 mm; most particles were smaller than 0.5 mm. In the United States bone particle sizes are limited in MSM for human consumption. According to the Code of Federal Regulations 9 CFR 319, "at least 98% of the bone particles present in MSM shall have a maximum size no greater than 0.5 mm in their greatest dimension and there shall be no bone particles larger than 0.85 mm in their greatest dimension" (Government, 2013).

The histological preparation technique also influences the outcome. Several authors (Branscheid, 2002; Josefowitz, 2008; Stenzel and Hildebrandt, 2006) provided overviews on actual staining techniques for bone tissue and their specificity and selectivity (Table 5). The detection of the typical bone morphology with osteocytes and canaliculi is thereby mandatory for bone diagnostics but may be already seen in the haematoxylin-eosin staining (Branscheid, 2002). Particularly suited for an automated evaluation process are stains in which bone tissue is differently coloured within an otherwise homogenously stained section background such as modified Kossa, Alizarin-S, Alizarin-red or modified van-Gieson staining (Hildebrandt and Josefowitz, 2007; Stenzel and Hildebrandt, 2006; Tremlova, 2000; Tremlova et al., 2006). However, since by these stains the bone particles remain calcified, the amount of fragments in the microscopic visual field may be reduced because the bone particles, due to their hardness, may leave the section during cutting (Branscheid, 2002; Stenzel and Hildebrandt, 2006). Therefore, Branscheid (2002) preferred techniques relying on decalcified bone tissues. Since in this technique bone apatite is solved and not available for staining, the author used

bone collagenous fibres as target tissue for staining. These fibres were stained with Sirus-red and detected in polarized light.

Table 5: Specific and selective detection of bone tissues (Branscheid, 2002).

Method	Principle	Characteristics
Specific Method		
Silver staining	Detection of calcium	Not reliable, non specific
Immunohistochemistry	Collagen	Time consuming, expensive, evaluation with fluorescence microscope needed, not suitable for heated material
Microradiography	Contact radiographic detection of Ca-apatite	Instrument seldom available
Selective method		
Polarisation microscopy	Detection of double breaks of Ca apatite (not decalcified) and collagen (decalcified)	Cheap, simple method only on decalcified samples
Morphology	e.g. Van Gieson staining	Not selective for bone particles, highly specific but often difficult for detection of osteocytes

Detection of bone particles, when present, can be a preliminary valuable tool within the framework of MSM examination. The presence of bone particles in MSM varies with the types of raw material used in the preparation and the processing method used. The presence of bone particles clearly indicates the presence of high pressure MSM. Low pressure MSM, which is currently also declared as MSM, contains fewer bone particles than high pressure MSM and in this respect is similar to fresh meat, minced meat and meat preparations. Thus, with the current MSM definition the distinction of MSM from fresh meat, minced meat and meat preparations only on the basis of bone particles content is not possible.

5.2.1.4. Cartilage

Cartilage and bone tissue are closely linked, especially in growing animals (articulation, rib, epiphyseal cartilage), which is the case for most of the animals slaughtered (Branscheid, 2002). Therefore, cartilage tissue is often included in the microscopic MSM detection. Several stains are used and include Astra blue, toluidine blue and others. However, in most cases the unique morphology of hyaline cartilage leads to diagnosis of cartilage.

Cartilage was detected in pressure based pork MSM in similar quantities as bone, between 0.3 and 1.9% (Bijker et al., 1983). In the study of Koolmees et al. (1986) pork MSM contained less cartilage particles (1 to 5%) than poultry (1 to 10%) and a tendency of press-type machines was seen to produce higher cartilage contents than auger-type separators. However, Bijker et al. (1985) pointed out that neither any relation could be established between cartilage and bone particle quantities nor could it be concluded that poultry MSM contained more cartilage than pork MSM. The authors concluded "…the cartilage content varies considerably depending on the nature of the raw materials processed and the adjustment and type of separation equipment".

The study of Pickering et al. (1995a) focused on the microscopical detection of hyaline cartilage after toluidine blue staining in MSM and in hand deboned meat from beef, pork, lamb, chicken and turkey from different carcase parts. Generally, MSM and hand deboned meat could be distinguished by the occurrence of cartilage particles. However, the results demonstrated also, that the amount of cartilage particles varied depending on the technology. Whereas pressure-piston separated meat from poultry contained this tissue regularly, hollow-drum/belt separators produced MSM with only one third of the samples containing cartilage particles. Additionally, necks of red meat species as raw material comprised no cartilage tissue. Thus, Pickering et al. (1995a) concluded that non-detection of hyaline

cartilage does not necessarily indicate the absence of MSM. Another limitation of this result is related to the detection of small amounts of MSM in meat mixtures where false negatives may arise (Pickering et al., 1995a). After validation the authors consider this as good screening method for MSM incorporation in products.

Similar to bone particles the regular detection of cartilage particles in histological slides indicates the presence of MSM. Since not all MSM types according to the present definition contain elevated amounts of cartilage particles, the distinction between MSM and fresh meat, minced meat and meat preparations is not consistently possible.

5.2.1.5. Bone marrow

Bone marrow was suggested as another suitable indicator to differentiate between MSM products irrespective of alteration or destruction of the bone structure (Branscheid and Judas, 2011; Field, 1999). The detection of bone marrow compounds would enable differentiation between MSM I useable for meat preparations [Annex III Sect. V Ch. III No 3 (d) of Reg. (EC) No. 853/2004] and MSM II, the use of which is limited to heat treated products [Annex III Sect. V Ch. III No 3 (e) of Reg. (EC) 853/2004]. With respect to microscopic analysis, Stenzel and Hildebrandt (2006) tried to use nuclei rich tissue compounds present between muscle fibres as an indicator for haematopoietic tissue. However, the attribution of these elements to bone marrow was not possible (Branscheid et al., 2009; Stenzel and Hildebrandt, 2006). Furthermore, red bone marrow shifts to fatty marrow with increasing age of the slaughtered animal, and also depends on bone types; hence, this parameter seems not suitable for all kinds of bones and age classes (Field, 1999).

5.2.1.6. Other tissues

The histological examination of MSM produced by turkey carcasses revealed the occurrence of renal structures (Josefowitz, 2008). According to Henckel et al. (2004) the higher risk of occurrence of material from other organs like kidney and lungs in poultry results from the raw material used. Whereas in mammals mostly individual bones are separated, in poultry MSM production is partly based on whole carcasses or back parts. The difficulty in removing these organs completely during standard automated evisceration procedures may lead to the appearance of the above-mentioned tissues in the derived material. However, as demonstrated by Josefowitz (2008), kidney particles were not detected in histological slides from discontinuous pressure-based turkey MSM in contrast to an auger separation process.

Detection of central nervous tissue has also been performed histologically, mostly in connection with evaluation for the presence of bovine spongiform encephalopathy risk material (Hafner et al., 2008; Kelley et al., 2000; Wenisch et al., 2000). Due to the fact that these techniques are limited to the detection of central nervous tissue, they would be applicable only to raw material for MSM production where such tissue could be found, namely cuts of the vertebral column. Therefore they are not universally applicable to distinguish MSM types or MSM from non-MSM.

The high dependence on the type of separated raw material excludes use of other tissues as general MSM technology or product type indicators.

5.2.2. Detection of morphological muscle structure changes

The impact of mechanical forces on structural properties of muscle foods has been studied by means of histological examination. The techniques used vary tremendously, since sample preparation, sectioning, staining and microscopic evaluation methods were more or less specifically adapted to the analytical question to be answered. Additionally the recognition and interpretation of morphological tissue distinctions require mostly expert knowledge of cell and tissue structural properties; thus, the development of an automated evaluation procedure is far from a simple task.

Morphological criteria to detect cell damage are described in section 4.2 of the present opinion. Mostly, clarity of striations, cell membrane disruptions, clarity of nuclei, and disorganization of nuclei as well as dispersed protein have been used.

Essentially, these manifestations of cell damage are also the basis of the computational analysis introduced by Sifre et al. (2009). The authors evaluated the integrity of muscle fibres in Calleja stained microscopic slides with the help of the nuclei position (migration to fibre centre or distribution in amorphous zones) and the breakdown of contractile proteins in the muscle fibre which may leak from the cell's interior through disrupted cell membranes and may form amorphous protein zones. According to the level of this destructuration in a sample, a MDI (meat destructuration indicator) value is calculated, being the ratio of destructured to total muscle fibre area. This calculation is performed by a computer algorithm based upon the image analysis of 150 images per sample scattered on three slides. This approach resembles the point counting technique in which large numbers of sample spots are analysed and results are based on frequency of occurrence. The MDI threshold for distinguishing meat from MSM was based on sensory assessment by a panel of 126 professionals**,** composed of: 45 judges from meat separation machine manufacturers; 56 from processed product manufacturers, distributors and scientists; and 25 from representatives of food regulatory bodies and consumer's associations. The threshold was set at 58.1%, according to the panel's list of judgements, but this value should be considered carefully, since, as pointed by the authors, this method, based on visual observation and touch, is empirical and lacks in precision due to the small number of judges. The authors determined the level of uncertainty of the method to be 3.2%. Correlation between MDI and chemical composition of the samples (collagen, calcium, fat, protein) was sought. Significant correlation (0.7) between fat cells and connective tissue on image segmentation and the level of free fat and collagen respectively was observed, but the correlation level between protein/nitrogen and calcium was low and not significant.

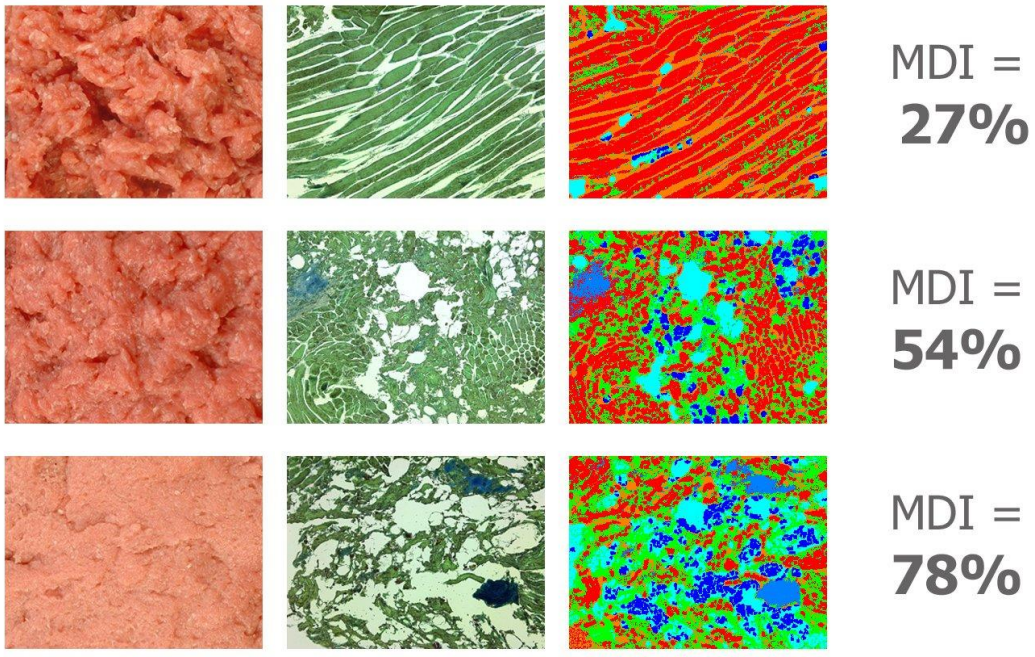

Figure 7: Macroscopic (histological Calleja staining), and virtual illustration of 3 different turkey raw meat samples and their MDI quantification result. The samples were processed in the laboratory in order to simulate 3 different levels of mechanical processing, minced meat, low pressure MSM and MSM (Source: Histalim).

The same authors further optimized this method especially considering the digital acquisition calibration focusing and the analysis workflow (Sifre et al., 2013).

With respect to this method, Branscheid et al. (2011) noted that the discrimination standard for meat and MSM is rather subjective and not entirely based on structural changes. Additionally, the unambiguousness of the Calleja staining was questioned: both the section thickness and the multi-fold of existing formulations may influence dye diffusion into the tissue and consequently the staining result. However, this method is a promising way for the distinction between MSM, minced meat and meat preparations.

Another method was presented by German authors (Branscheid et al., 2012; Branscheid and Troeger, 2012), and was based upon the above mentioned four characteristics of cell integrity. The authors stained sections from minced or auger-sieve separated poultry meat with toluidine blue and classified the observed structure damages in four standards. Standards 1 and 2 (without alteration and comminuted fibres with normally arranged nuclei) were assessed as effects occurring in all deboning techniques inclusive of minced meat. When serious technical effects occur during deboning, standard 3 damages were observed which include changes of the nuclei arrangements and metachromatic colour changes due to squeezed cartilage matrix. Complete structure dissolution with intense colouring and metachromatic effects was designated as standard 4, which was only seen with MSM. The authors suggested creation of limits for the frequency of occurrence of standards 3 and 4 in the slide: for example > 20% standard 3 or > 5% standard 4 would indicate a highly damaged muscle fibre structure thus resulting in an imperative labelling as MSM. A major drawback for the use of this method is the lack of objective validation, since the authors of this study validated it only by double histological evaluation of the sections.

A similar study was performed in 2011 by Groves (2011) evaluating chicken, turkey and pork samples processed with conventional mincers as well as with hollow-drum/belt, auger-sieve and press-type separation machines using different pressures. The project was contracted by the Food Standards Agency with the intention to help local authorities and public analysts in the UK to assess whether a particular meat ingredient produced by mechanical removal of residual meat from bones falls within the definition of MSM, also known as mechanically recovered meat (MRM). The project looked at whether a simple microscopy protocol could be used to differentiate between hand-deboned meat that has been mechanically treated (such as mincing/chopping), and other MSM types. Cryostat sections were stained in toluidine blue and viewed by light microscopy, paying attention to the section colour, presence and amount of muscle blocks, presence and condition of muscle fibres (level of intact muscle fibres, visible banding extent/amount of dispersed protein) as well as presence and amount of connective tissue, hyaline cartilage and spaces in the section (Table 6). The metachromatic effects described by Branscheid et al. (2012) were also documented in this study for all high pressure separation techniques. The results showed clear differences in appearance and muscle integrity between minced, low pressure MSM and high pressure MSM. These differences and the sample type were identified easily in a blind trial by scientists with minimal training in microscopy and provided an overview of the key features elaborated by Groves (2011). Nevertheless to assess whether an ingredient qualifies as meat or as MSM is based on a subjective decision made by considering the results from the microscopy assessment, together with the history and condition of the sample (Groves, 2011). Additionally, dispersed protein also appears in minced meat to different extents depending on the raw material (chilled/frozen) and the technology used (single/double mincing, mincer type, etc.; Upmann, unpublished data).

Table 6: Summary of key features of muscle fibres in minced meat, hand deboned meat, low pressure MSM and high pressure MSM (Groves, 2011).

Product	Colour in toluidine blue	Integrity of muscle fibres	Presence of muscle blocks	Banding of fibres	Hyaline Cartilage present	Dispersed protein
Minced Meat	Pink / purple	Mostly intact	Easily visible	Visible at high magnification	Not usually present but some fragments of hyaline cartilage or bone might be present in hand deboned meat	Some dispersed protein present- usually less than 20% of the area
Hand Deboned Meat	Pink / purple / blue	Mostly intact	Easily visible	Visible at high magnification	Fragments of bone sometimes found	Partly present at low fraction of sample (<20%)
Low pressure MSM	Similar to minced but might be more lilac depending on level of connective tissue	Many intact but increased fragmentation of fibres	Present but less so than in minced meat	Some banding visible at high magnification	Often present	Considerable amount of dispersed protein present. Varies with meat type and machine
High pressure MSM	Increase in green / blue colouration often seen	Very little seen	None seen	Sometimes seen within the matrix	Usually present	Mostly dispersed protein with little intact muscle structure visible

In an earlier study concerning poultry meat, Branscheid et al. (2009) used the same staining method in combination with polarized light for the detection of muscle structure changes. Less colouring of the sarcoplasm, pale or lacking transverse striation and nuclei destruction were seen with press-type MSM, and a loss of double refraction with only fragmentary striation as the most indicative phenomenon. In contrast, hollow-drum/belt separator product as well as minced meat did not show such damage. However, it is not yet clear if this method is also applicable to red meat species.

Recently, Henckel et al. (2011) outlined a method using antibodies in order to evaluate muscle fibre damage. In comparison to a haematoxylin-eosin staining the authors applied firstly antibodies directed towards laminin since the degradation of muscle fibres apparently affects the antibody's binding properties to laminin. Attention should be paid to the fact that laminin also exists in the basal membranes of other tissues so that tissue structures must also be considered. This laminin-staining was combined with an antibody-based myosin staining giving a good indication of the amount of muscle tissue. An automated image analysis system was used for image capture and data analysis. According to Henckel et al. (2011), this method was suited to detect the amount of muscle fibre damage which was reasonably lower in auger-sieve separated and in emulsified meat (<28%) than in hollow-drum/belt separated and manually deboned and minced and coarsely chopped meat (>40%). However, the methodological aspects are not fully described and the applicability of the method to other meat animal species is not yet validated. Additionally, laboratory equipment for immunohistology differs significantly from that used for conventional histological methods described above and the availability of the laminin antibody is unclear.

As a conclusion, microscopic examination of tissue changes appears promising as a tool for the differentiation of MSM types, minced meat and meat preparations, but objective threshold values are not yet established. All four methods currently available for the description of morphological muscle

structure changes by Sifre, Branscheid, Groves and Henckel have some limitations. The method proposed by Sifre seems promising in itself but the threshold set by the panel of professionals is too empirical and subjective and insufficiently validated; the method proposed by Groves is not quantitative, and the methods proposed by Branscheid are not properly validated yet. In the method of Henckel some fundamental data concerning its general applicability are lacking.

5.3. Molecular methods

Some molecular techniques have been studied as potential methods for detecting and differentiating MSM from hand deboned meat (e.g. electrophoretic techniques, proteomics, metabolomics, etc.), however these are still at experimental level without proper validation and in practice complexity and cost may limit their application.

5.3.1. Electrophoretic techniques

Electrophoretic techniques have been used to separate meat proteins by SDS-PAGE, capillary gel electrophoresis or isoelectric focusing followed by multivariate data analysis (Skarpeid et al., 2001b). Differences in the relative concentrations of several proteins were observed, with haemoglobin content higher in marrow than in meat, and hence also higher in MSM than hand deboned chicken breast meat (HDM). On the other hand, HDM was characterized by higher amounts of actin, myosin and myoglobin.

Capillary gel electrophoresis was used as a method for differentiating between raw mechanically recovered chicken meat and HDM. Differences in the relative peak areas within the profiles obtained distinguished raw MSM from raw HDM; specifically, that of haemoglobin was higher in MSM. Using the peak area of haemoglobin and its ratio to other peaks, the technique was tested using composite MSM-HDM mixtures. The results suggest that it is possible to differentiate mixtures containing 7.5% MSM from that of 0% MSM using the capillary gel electrophoresis method (Day and Brown, 2001).

5.3.2. Proteomics

Since single dimensional gel electrophoresis may not provide sufficient resolution, and less abundant, but potentially significant, proteins may be missed when SDS-PAGE alone is used, proteomics relies on two-dimensional (2D) gel electrophoresis. An alternative approach to 2D gel electrophoresis is Off-Gel™ isoelectric focusing electrophoresis, where proteins are separated according to their isoelectric point (pI) values, then recovered from solution and can be directly used for SDS-PAGE separation, enzyme digestion, crystallization or mass spectrometry (Michel et al., 2003).

Intact proteins were extracted from raw meat and then analyzed with OFF-GEL electrophoresis followed by SDS-PAGE and identification of potential markers by nano-LC-MS/MS. It was shown that it is possible to extract, separate and identify key proteins from processed meat material. Potential chicken mechanically recovered meat markers - haemoglobin subunits and those similar to myosin-binding protein C - were also identified.

5.3.3. Metabolomics

Metabolite profiling (metabolomics) is a method for biomarker detection in biological samples. It focuses on relative quantification of as many as possible metabolites in a biological material followed by application of chemometric methods for selection of compounds that are characteristic in that material. A pilot study (Surowiec et al., 2011a) of metabolite profiling in meat samples performed GC–MS followed by partial least squares analysis to find the best extraction method for meat metabolome, which was then tested on extracts from selected chicken hand deboned and MSM samples, on pork samples and on hand deboned meat and MSM samples from different sources. The compounds were tentatively identified by comparison of their retention indices and MS spectra and appeared to belong to a variety of chemical classes, with the most common being fat-related compounds. The selected compounds cannot serve as markers alone, but the proposed methodology can be used for multivariate sample classification. The results showed that it was not possible to select

few specific biomarkers, but class differentiation and proper classification of new samples were obtained using all variables (compounds).

5.4. Immunological methods

Antibodies were raised against a low molecular weight fraction of chicken bone marrow proteins and an enzyme-linked immunosorbent assay (ELISA) was developed. The system was used to test for the presence of mechanically recovered meat in a range of product types, from raw chicken meat to heat processed samples. The results showed that it is possible to raise antibodies to chicken bone marrow proteins which show a strong reactivity with chicken and turkey MSM but show little reaction with extracts of MSM and hand deboned meat of other common meat species. However, blood, skin and soya all affected the accuracy of the ELISA (Pickering et al., 1995b).

Other trials focused on the detection of cartilage glycosaminoglycan (keratan sulphate) by immunodiffusion analysis using anti-keratan sulfate monoclonal antibody (IgM) in meat products containing mechanically separated chicken meat (MSCM) having cartilage particles (Nakano et al., 2012). The immunodiffusion test appears to be a simple sensitive specific method for qualitative analysis of keratan sulfate, but should be used in combination with other methods.

5.5. Combinations of methods and/or tests

Since no single method has been identified as effective in measuring a parameter that would efficiently distinguish different types of MSM from non-MSM, the potential of using combined tests based on histological and other physical or chemical analyses may be considered. Such an approach would need setting and validation of threshold values for the selected parameters. For example, a multistep sequential analytical approach could be designed to answer questions based on the criteria included in the definition of MSM in Reg. (EC) No. 853/2004 and on some other parameter measures. The results could then be used in the assignment of a product to a meat category . An example of such an approach could be the following:

i) Is the muscle fibre structure of the product lost or modified (validated test and threshold values are needed)?

ii) Are bone particles present in the product, in which number per unit of volume and of which size (validated test and threshold values are needed)?

iii) What is the calcium and the cholesterol content of the product (validated test and threshold values are needed)?

Other combination analyses could also be considered.

6. Analysis of chemical composition parameters for hand deboned meat and MSM

6.1. Collection of published data for evaluation

A database on chemical characteristics of hand deboned meat (HD) and MSM from poultry and pork was developed with 338 entries by screening 74 scientific papers. The data on chemical characteristics included moisture, protein, fat, ash, calcium, iron, cholesterol and collagen, and they were expressed in percentage or in mg/100 g product.

6.2. Data description and limitations

Data were retrieved from different studies where samples from different species and raw materials as well as analytical methods had been used. The data used were from studies not systematically designed for the purpose of this analysis, so it was not possible to compare products from the same animal species and the same raw material.

The entries in the database could be univocally categorised only as MSM or hand deboned meat. Extraction of information about different types of MSM (low or high pressure) was not possible from the literature analysed, since no clear and detailed information was provided about the processing conditions used. In studies about MSM the commercial type of machinery used is generally indicated but the values of pressure applied are generally absent. Moreover most of the machineries available on the market may be set for high or low pressure MSM production, according to the type of raw material to be processed. This means that the distinction between different types of MSM is not possible using the currently available published data. Therefore, the analysis of these data allows differentiation only between MSM and non-MSM (fresh meat, hand deboned meat, minced meat, meat preparations).

6.3. Data analysis

The chemical characteristics of hand deboned and MSM meat were compared both graphically and statistically. For graphical comparison scatterplots and box-plots were used. For statistical comparison F-tests were applied to evaluate the variances followed by a t-test for equal or unequal variances depending on the results of the F-tests.

The possible influence of animal species and animal parts were checked (see Table 24 in Appendix B) and the results of the analysis of variance for calcium showed that only the processing method (hand deboned meat vs. MSM) is significant ($P<0.05$). Animal species and parts are not significant ($P>0.05$).

Furthermore binary logistic regression analysis was performed in order to identify the probability for a product to be classified as MSM based on calcium content. Hand deboned meat and MSM were assigned values of 1 or 0, respectively. Data were fitted to a logistic regression model using Minitab software (Minitab Inc. PA, USA). For modelling purposes a logarithmic transformation was used for the calcium concentration. The model was of the form shown in the following equation.

$$\text{Logit}\,(P) = a_0 + a_1 \log(C) + a_2 (\log(C))^2$$

where, Logit (P) is an abbreviation of ln[P/(1-P)], P is the probability (in the range of 0-1) for a product to be classified as MSM meat, a_i are coefficients to be estimated, and C is the calcium concentration. The logistic regression model was derived using the logistic regression of the Minitab software. The automatic variable selection option with a stepwise selection method was used to choose the most significant effects ($P<0.05$). The predicted interfaces for P=0.1, 0.5 and 0.9 were calculated using Microsoft Excel.

6.4. Presentation of results

The results of the analysis are reported below. In the Figures 8-16 the values for the parameters analysed are displayed, divided in hand deboned meat (HD) and MSM and in Tables 7-22 the statistics

of content data of HD meat and MSM retrieved from the publications analysed and F-Test two-sample for variances for each parameter are shown.

6.4.1. Protein

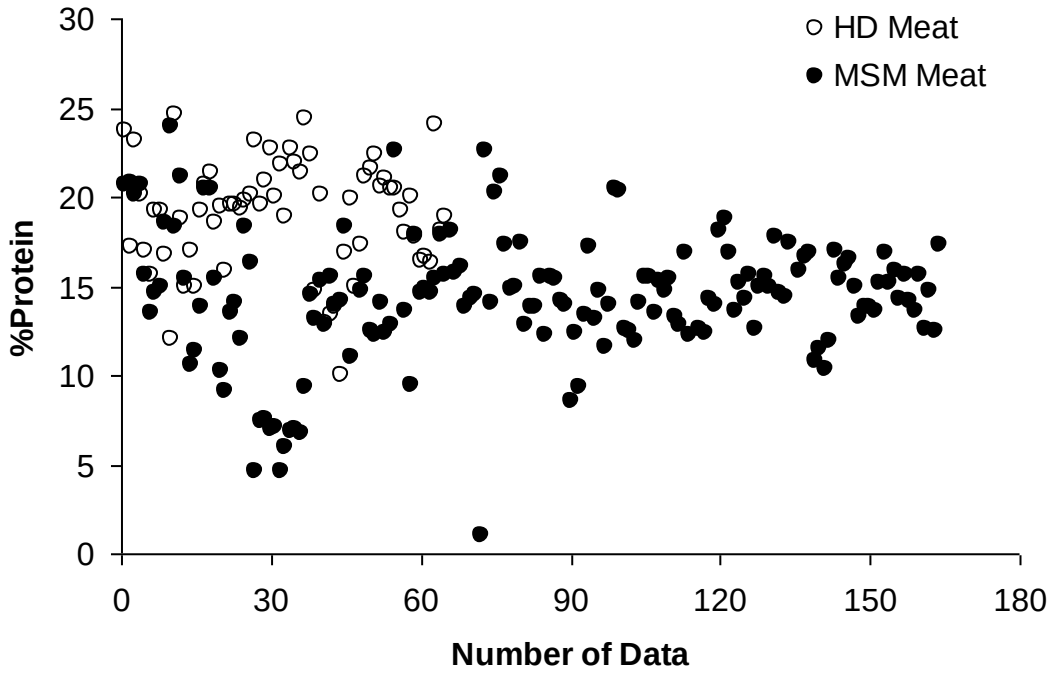

Figure 8: Scatterplot showing the protein content data (%) for HD meat and MSM retrieved from the publications analysed.

Table 7: Statistics of protein content data (%) of HD meat and MSM retrieved from the publications analysed.

	HD	MSM
Mean	19.86	15.23
SD	7.81	7.13
Min	9.96	1.00
Max	77.30	84.30
5th Percentile	13.50	7.10
95th Percentile	23.92	21.10

Table 8: F-Test Two-Sample for Variances (protein).

	HD	MSM
Mean	19.86	15.23
Variance	61.00	50.84
Observations	66	158.00
Df	65.00	157.00
F	1.20	
P(F<=f) one-tail	0.18	
F Critical one-tail	1.39	

Table 9: t-Test: Two-Sample Assuming Equal Variances (protein).

	HD	MSM
Mean	19.86	15.23
Variance	61.00	50.84
Observations	66	158
Pooled Variance	53.81	
Hypothesized Mean Difference	0	
df	222	
t Stat	4.31	
P(T<=t) one-tail	1.23E-05	
t Critical one-tail	1.652	
P(T<=t) two-tail	2.46E-05	
t Critical two-tail	1.971	

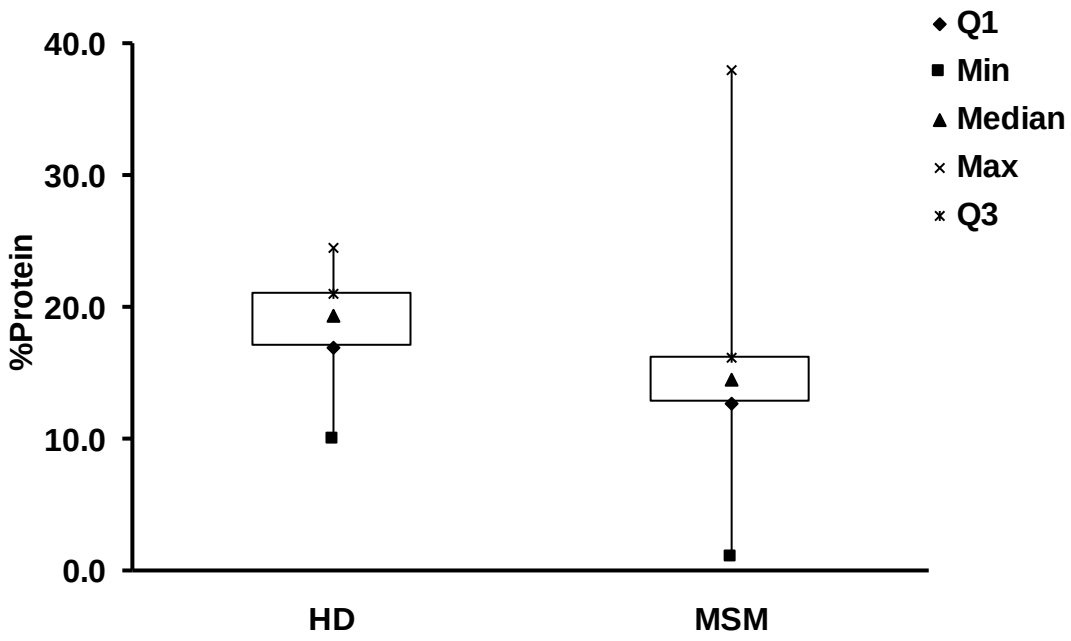

Figure 9: Scatterplot showing protein content data (%) for HD meat and MSM retrieved from the publications analysed.

The analysis of data for protein content included 66 data points for HD meat and 158 data points for MSM. The mean and s.d. values were 19.86 and 7.8 for HD meat and 15.2 and 7.13 for MSM, respectively. The F-test showed equal variances between the variables (P(F<=f) one-tail>0.05). The t-Test for two-sample assuming equal variances showed that protein content in HD meat and MSM differ significantly (P(T<=t) two-tail<0.05). However, as shown in the scatterplot and the boxplot there is a significant overlapping of the protein content between HD meat and MSM indicating that protein content is not an appropriate indicator for classifying a product as MSM.

6.4.2. Ash

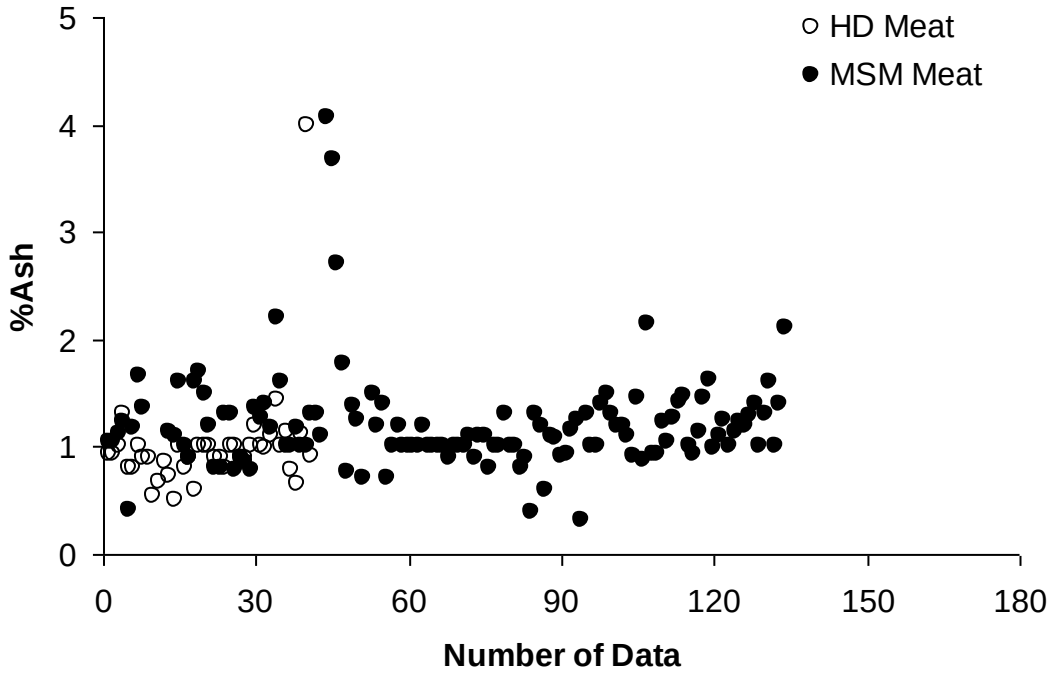

Figure 10: Scatterplot showing ash content data (%) for HD meat and MSM retrieved from the publications analysed.

Table 10: Statistics of ash content data (%) of HD meat and MSM retrieved from the publications analysed.

	HD	MSM
Mean	0.997	1.639
SD	0.515	2.497
Min	0.500	0.320
Max	4.000	20.196
5th Percentile	0.600	0.777
95th Percentile	1.300	3.049

Table 11: F-Test Two-Sample for Variances (ash).

	HD	MSM
Mean	0.997	1.639
Variance	0.265	6.234
Observations	41	134
df	40	133
F	0.0425	
P(F<=f) one-tail	0	
F Critical one-tail	0.6368	

Table 12: t-Test: Two-Sample Assuming Unequal Variances (ash).

	HD	MSM
Mean	0.997	1.639
Variance	0.265	6.234
Observations	41	134
Hypothesized Mean Difference	0	
df	162	
t Stat	-2.786	
P(T<=t) one-tail	0.003	
t Critical one-tail	1.654	
P(T<=t) two-tail	0.006	
t Critical two-tail	1.975	

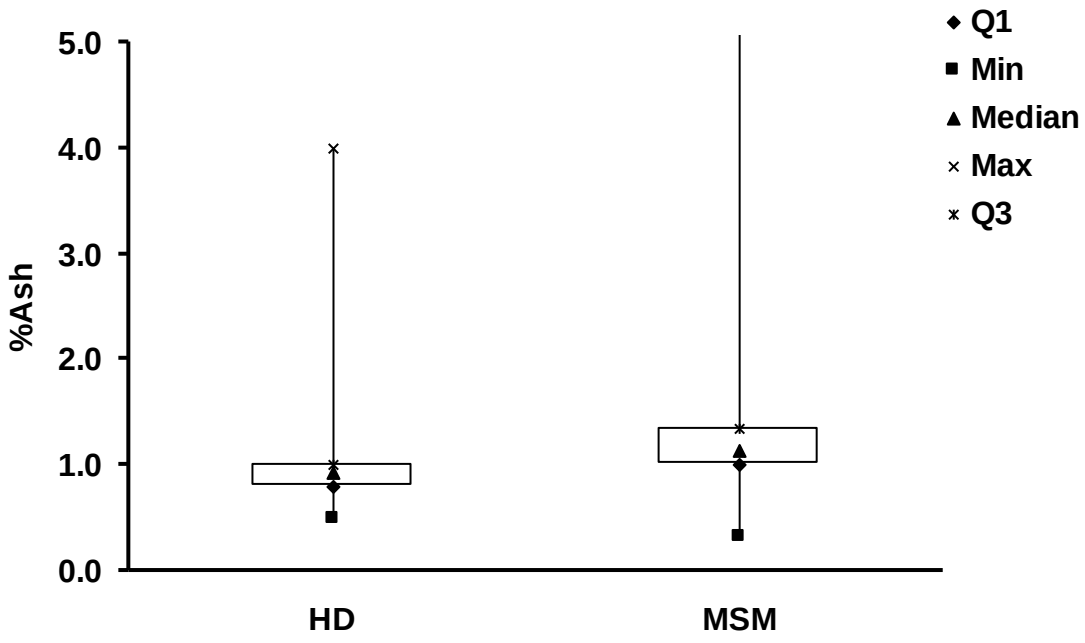

Figure 11: Scatterplot showing ash content data (%) for HD meat and MSM retrieved from the publications analysed.

The analysis of data for ash content included 41 data points for HD meat and 134 data points for MSM. The mean and s.d. values were 1.0 and 0.51 for HD meat and 1.6 and 2.5 for MSM, respectively. The F-test showed unequal variances between the variables (P(F<=f) one-tail>0.05). The t-Test for two-sample assuming unequal variances showed that ash content in HD meat and MSM differ significantly (P(T<=t) two-tail<0.05). However, as shown in the scatterplot and the boxplot there is a significant overlapping of the ash content between HD meat and MSM indicating that ash content is not an appropriate indicator for classifying a product as MSM.

6.4.3. Cholesterol

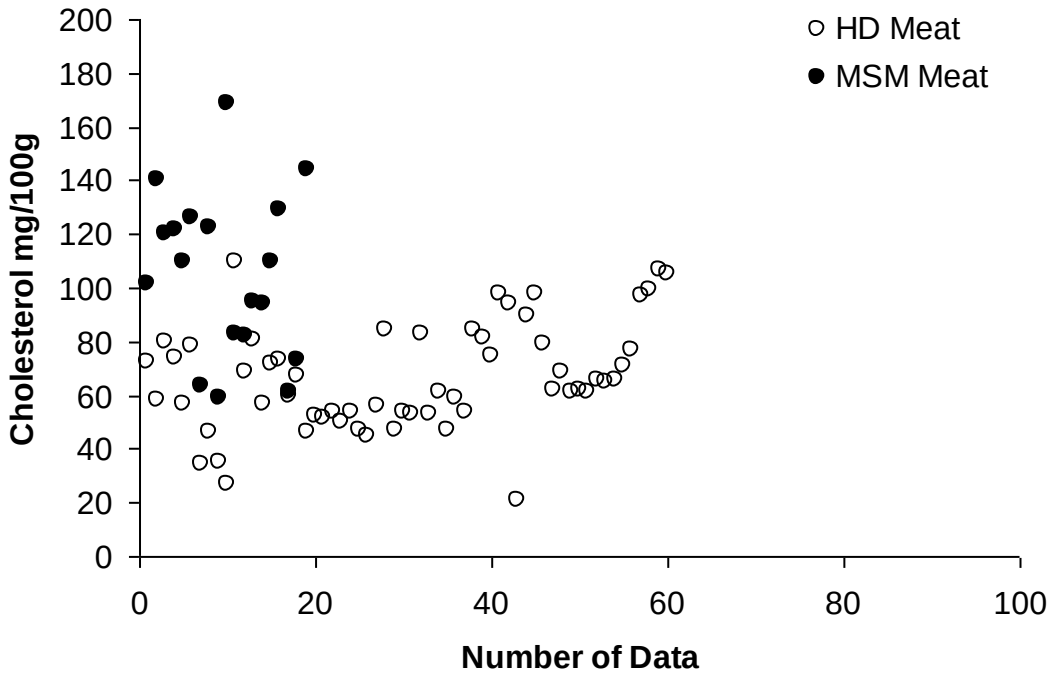

Figure 12: Scatterplot showing cholesterol content data (mg/100 g) for HD meat and MSM retrieved from the publications analysed.

Table 13: Statistics of cholesterol content data (mg/100 g) of HD meat and MSM retrieved from the publications analysed.

	HD	MSM
Mean	66.428	105.465
SD	19.520	30.523
Min	20.770	58.750
Max	110.000	168.890
5th Percentile	35.000	60.775
95th Percentile	99.000	146.489

Table 14: F-Test Two-Sample for Variances (cholesterol).

	HD	MSM
Mean	66.428	105.465
Variance	381.048	931.660
Observations	61	19
df	60	18
F	0.409	
P(F<=f) one-tail	0.005	
F Critical one-tail	0.562	

Table 15: t-Test: Two-Sample Assuming Unequal Variances (cholesterol).

	HD	MSM
Mean	66.428	105.465
Variance	381.048	931.660
Observations	61	19
Hypothesized Mean Difference	0	
df	23	
t Stat	-5.250	
P(T<=t) one-tail	1.25606E-05	
t Critical one-tail	1.714	
P(T<=t) two-tail	2.51213E-05	
t Critical two-tail	2.069	

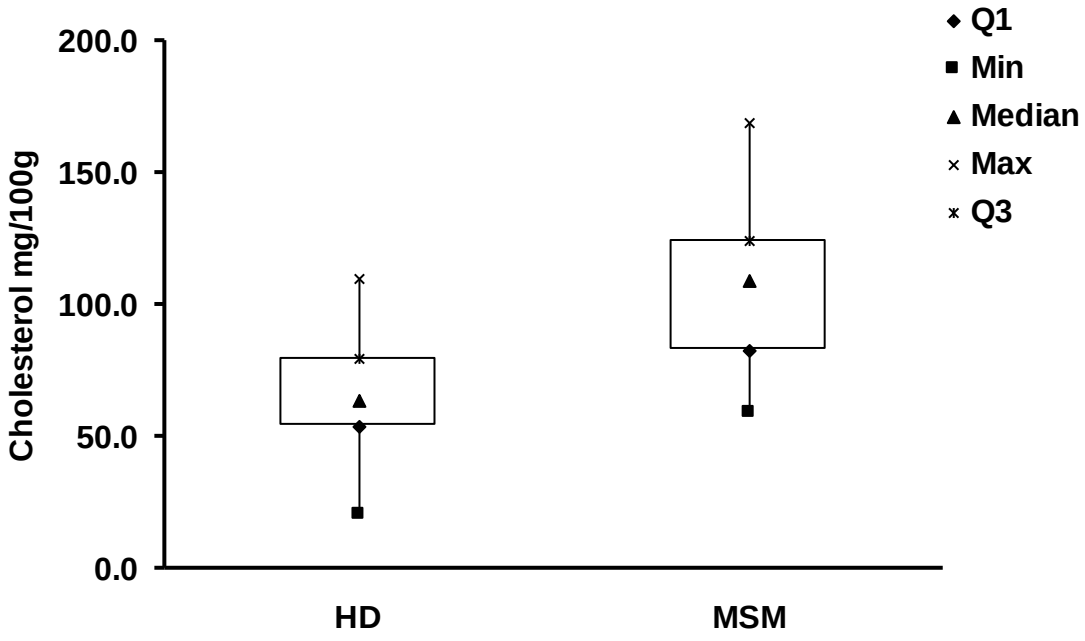

Figure 13: Scatterplot showing cholesterol content data for HD meat and MSM retrieved from the publications analysed.

The analysis of data for cholesterol content included 61 data points for HD meat and 19 data points for MSM. The mean and s.d. values were 66.4 and 19.5 for HD meat and 105.5 and 30.52 for MSM, respectively. The F-test showed unequal variances between the variables (P(F<=f) one-tail>0.05). The t-Test for two-sample assuming unequal variances showed that cholesterol content in HD meat and MSM differ significantly (P(T<=t) two-tail<0.05). As shown in the scatterplot and the boxplot there is no significant overlapping of the cholesterol content between HD meat and MSM indicating that cholesterol content could be used as an appropriate indicator for classifying a product as MSM. However, the available data on cholesterol content in MSM is limited and further research for validations is required.

6.4.4. Iron

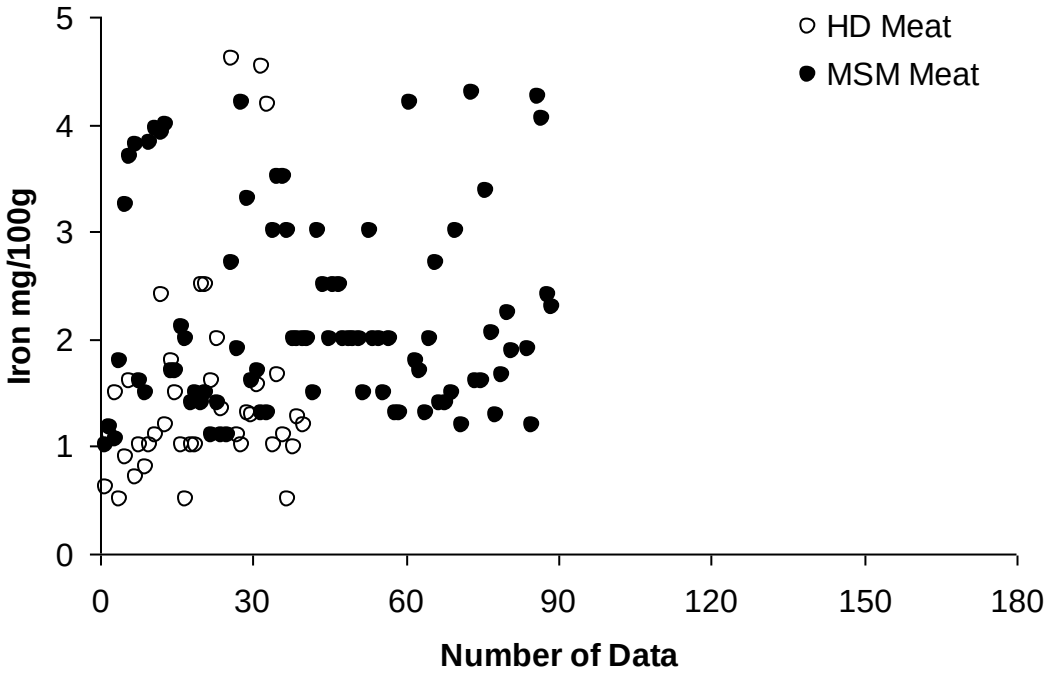

Figure 14: Scatterplot showing iron content data (mg/100 g) for HD meat and MSM retrieved from the publications analysed.

Table 16: Statistics of iron content data (mg/100 g) of HD meat and MSM retrieved from the publications analysed.

	HD	MSM
Mean	1.595	2.746
SD	1.148	3.208
Min	0.500	1.000
Max	5.300	22.600
5th Percentile	0.500	1.128
95th Percentile	4.530	4.280

Table 17: F-Test Two-Sample for Variances (iron).

	HD	MSM
Mean	1.595	2.746
Variance	1.318	10.294
Observations	40	89
df	39	88
F	0.128	
P(F<=f) one-tail	1.39E-10	
F Critical one-tail	0.622	

Table 18: t-Test: Two-Sample Assuming Unequal Variances (iron).

	HD	MSM
Mean	1.595	2.746
Variance	1.319	10.294
Observations	40	89
Hypothesized Mean Difference	0	
df	123	
t Stat	-2.986	
P(T<=t) one-tail	0.0017	
t Critical one-tail	1.657	
P(T<=t) two-tail	0.003	
t Critical two-tail	1.979	

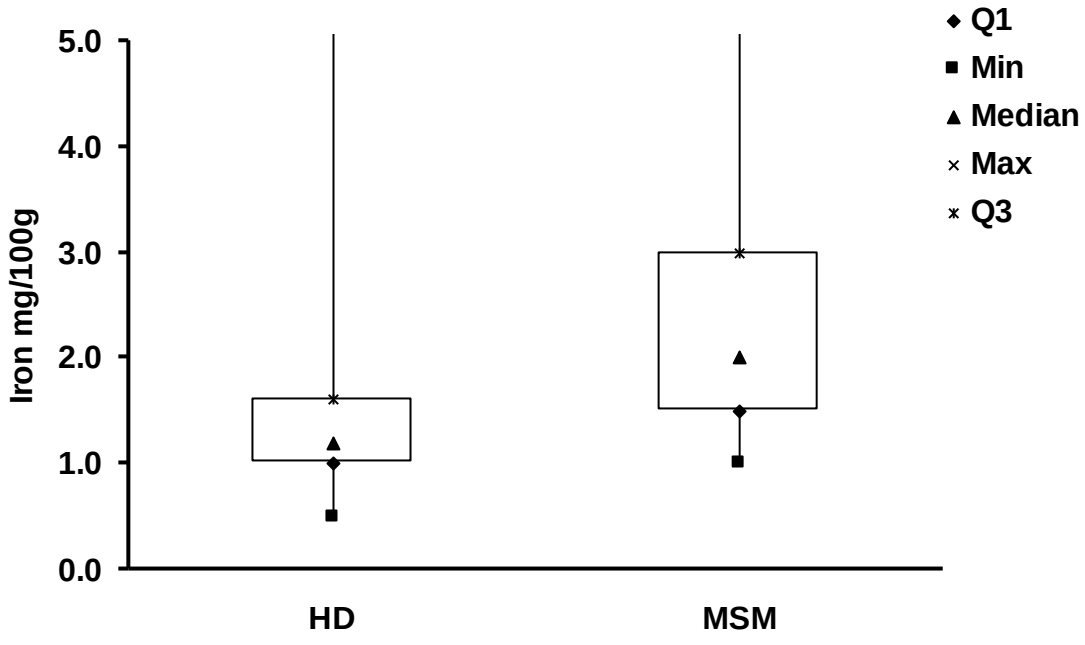

Figure 15: Scatterplot showing iron content data for HD meat and MSM retrieved from the publications analysed.

The analysis of data for iron content included 40 data points for HD meat and 89 data points for MSM. The mean and s.d. values were 1.6 and 1.15 for HD meat and 2.7 and 3.21 for MSM, respectively. The F-test showed unequal variances between the variables (P(F<=f) one-tail>0.05). The t-Test for two-sample assuming unequal variances showed that iron content in HD meat and MSM differ significantly (P(T<=t) two-tail<0.05). However, as shown in the scatterplot and the boxplot there is a significant overlapping of the iron content between HD meat and MSM indicating that iron concentration is not an appropriate indicator for classifying a product as MSM.

6.4.5. Calcium

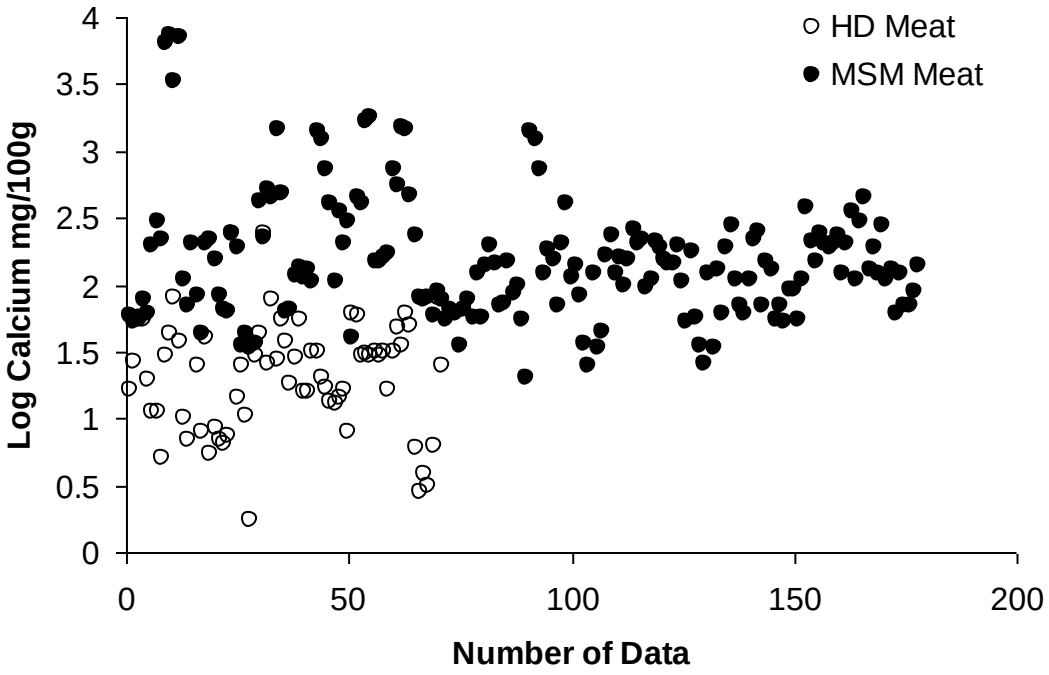

Figure 16: Scatterplot showing calcium content data (mg/100 g) for HD meat and MSM retrieved from the publications analysed.

Table 19: Statistics of calcium content data (mg/100 g) of HD meat and MSM retrieved from the publications analysed.

	HD	MSM
Mean	27.96	358.26
SD	34.17	980.66
Min	0.50	20.30
Max	241.90	7410.00
5th Percentile	3.07	40.90
95th Percentile	62.70	1419.25

Table 20: F-Test Two-Sample for Variances (calcium, mg/100 g).

	HD	MSM
Mean	27.957	358.261
Variance	1167.505	961702.948
Observations	59	166
df	58	165
F	0.0012	
P(F<=f) one-tail	0	
F Critical one-tail	0.687	

Table 21: t-Test: Two-Sample Assuming Unequal Variances (calcium, mg/100 g).

	HD	MSM
Mean	27.957	358.260
Variance	1167.505	961702.948
Observations	59	166
Hypothesized Mean Difference	0	
df	166	
t Stat	-4.332	
P(T<=t) one-tail	1.27448E-05	
t Critical one-tail	1.654	
P(T<=t) two-tail	2.54896E-05	
t Critical two-tail	1.974	

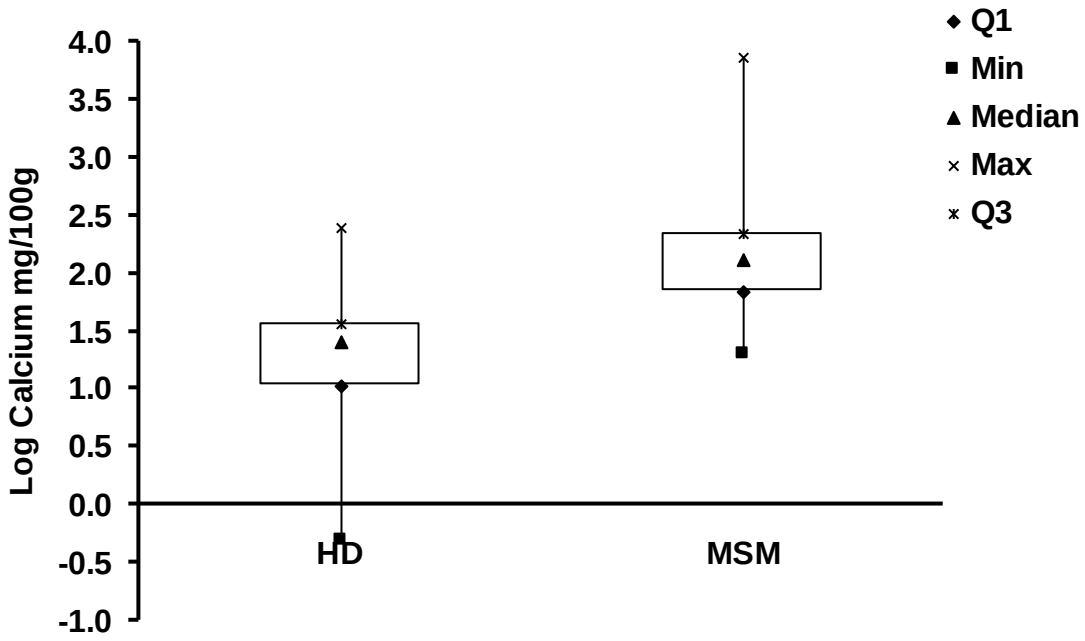

Figure 17: Scatterplot showing calcium content data for HD meat and MSM retrieved from the publications analysed.

The analysis of data for calcium content included 59 data points for HD meat and 166 data points for MSM. The mean and s.d. values were 28.0 and 34.17 for HD meat and 358.26 and 980.66 for MSM, respectively. The F-test showed unequal variances between the variables (P(F<=f) one-tail>0.05). The t-Test for two-sample assuming unequal variances showed that calcium content in HD meat and MSM differ significantly (P(T<=t) two-tail<0.05). As shown in the scatterplot and the boxplot there is no significant overlapping of the calcium content between HD meat and MSM. In general, the analysis of the results showed that calcium content is the most appropriate indicator for classifying a product as MSM.

6.5. Binary Logistic Regression

The parameter estimates and statistics of the logistic regression model with non significant (P>0.05) effects removed, are shown in Table 22.

Table 22: Parameter estimates and statistics of the logistic regression model.

Link Function: Logit

Response Information

Variable	Value	Count	
R	1	178	(Event)
	0	71	
	Total	249	

Logistic Regression Table

Predictor	Coef	SE Coef	Z	P	Odds Ratio	95% CI Lower	95% CI Upper
Constant	-18.650	4.030	-4.63	0.000			
C	15.861	4.011	3.95	0.000	7.73E+06	2976.68	2.01E+10
C^2	-2.5947	0.9843	-2.64	0.008	0.07	0.01	0.51

Log-Likelihood = -64.885

Test that all slopes are zero: G = 167.906, DF = 2, P-Value = 0.000

Goodness-of-Fit Tests

Method	Chi-Square	DF	P
Pearson	258.741	178	0.000
Deviance	110.903	178	1.000
Hosmer-Lemeshow	13.618	8	0.092

Table of Observed and Expected Frequencies:
(See Hosmer-Lemeshow Test for the Pearson Chi-Square Statistic)

					Group						
Value		1	2	3	4	5	6	7	8	9	10	Total
1												
	Obs	0	3	12	18	22	27	24	24	23	25	178
	Exp	0.1	3.9	11.6	19.1	21.6	25.4	23.2	23.6	24.7	24.9	
0												
	Obs	24	22	13	7	3	0	0	0	2	0	71
	Exp	23.9	21.1	13.4	5.9	3.4	1.6	0.8	0.4	0.3	0.1	
	Total	24	25	25	25	25	27	24	24	25	25	249

Measures of Association:
(Between the Response Variable and Predicted Probabilities)

Pairs	Number	Percent	Summary Measures	
Concordant	11874	94.2%	Somers' D	0.88
Discordant	738	5.8%	Goodman-Kruskal Gamma	0.88
Ties	26	0.2%	Kendall's Tau-a	0.36
Total	12638	100.0%		

The concordance index, the Hosmer—Lemeshow goodness-of-fit statistic and the maximum rescaled R-square statistic were used as measures of goodness of fit of the model developed. As determined by the concordance index, the degree of agreement between the predicted probabilities and the observations was 94.2% concordant and 5.8% discordant. The Hosmer—Lemeshow goodness-of-fit statistic was 13.618 (Chi-Square with 8 degrees of freedom; P=0.092).

The following graph shows the probability for a product to be classified as MSM based on the calcium content (Figure 18).

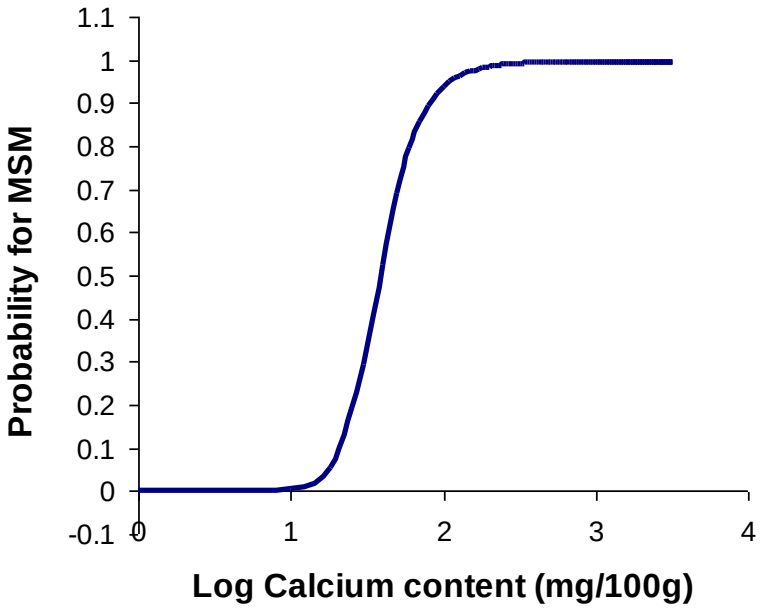

Figure 18: Probability for a product to be classified as MSM based on the calcium content based on the logistic regression model.

The calcium content corresponding to probabilities from 0.05 to 0.99 for a product to be classified as MSM as predicted by the binary logistic regression model are shown in Table 23.

Table 23: Calcium contents corresponding to probabilities from 0.05 to 0.99 for a product to be classified as MSM as predicted by the binary logistic regression model.

Calcium content (mg/100 g)	Probability to classified as MSM
17.5	0.05
21	0.10
25	0.17
30	0.29
39	0.50
45	0.62
50	0.69
55	0.75
60	0.79
65	0.83
70	0.85
75	0.87
81.5	0.90
100	0.936
111	0.95
280	0.99

The model was incorporated into an Excel application (Figure 19) where the user can easily introduce the calcium content and estimate the probability for a product to be classified as MSM.

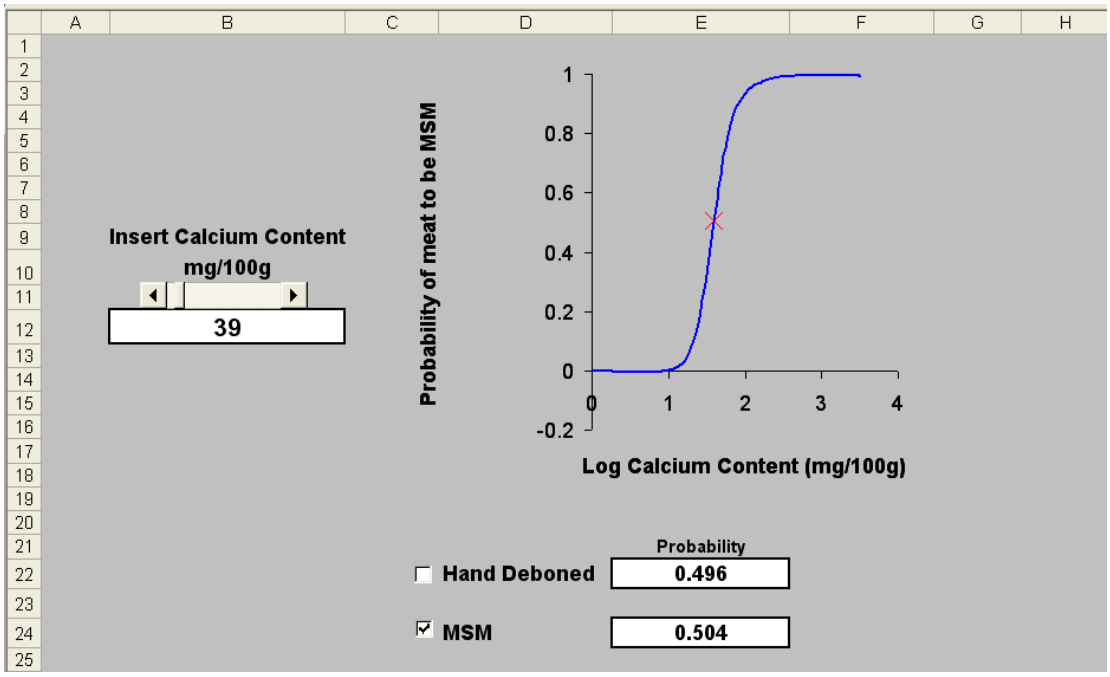

Figure 19: Excel application for estimating the probability for a product to be classified as MSM on the basis of calcium content.

6.6. Conclusions from data analysis

The analysis of the available data in the literature showed statistically significant difference (P(T<=t) two-tail<0.05) between HD meat and MSM for all tested chemical characteristics (protein, ash, calcium, iron, cholesterol).

Analysis by animal species showed no major differences in the calcium content between poultry and pork. Therefore, all data were combined and analysed.

Due to overlapping of data, the discriminatory power between HD meat and MSM provided by protein, ash and iron contents is low, thus indicating that these characteristics are not good indicators for classifying a product as MSM.

No significant overlapping was observed for cholesterol content between HD meat and MSM indicating that cholesterol could be used as an appropriate indicator for classifying a product as MSM. However, the available data on cholesterol content in MSM are limited and further research for validation is required.

A binary logistic regression analysis was performed in order to identify the probability for a product to be classified as MSM based on calcium content. According to the binary logistic regression analysis, calcium was found to be the most appropriate indicator for classifying a product as MSM. The analysis showed that calcium content of 21, 39 and 81.5 and 100 mg/100 g corresponded to probabilities of 0.1, 0.5, 0.9 and 0.936 for a product to be classified as MSM.

The performance of the binary logistic regression model was tested against unpublished data from hand deboned meat and MSM samples provided by the industry. The model provided probability under 50% to be classified as MSM for 95.8% of the hand deboned samples based on their calcium content, indicating a good performance of the model in correctly classifying hand deboned samples. For high pressure MSM products the probability provided by the model was above 50% for 92.6% of the samples. For products characterised by the industry with a meat destructuration index <58.1% according to the test proposed by Sifre et al. (2009) (see chapter 5.2.2), the model provided a probability of below 50% to be classified as MSM for 78.0% of the samples. For other samples from poultry wishbone and poultry carcasses produced with the belt-drum system (a method that generally applies low pressure), the model provided a probability of below 50% to be classified as MSM for 93.2% of the samples, based on their calcium content.

It needs to be noted that the data used in the above analysis were not collected for the purpose of this analysis. In particular, in most cases, hand deboned and MSM samples were derived from different raw materials. Specifically designed studies for the collection of data on calcium contents in hand deboned and MSM products derived from the same raw material and taking into account different animal species and body parts could lead to an improved approach for MSM identification.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

General conclusion

- Based on the current EU Regulation, low and high pressure MSM are defined according to the alteration of bone structure and calcium content. The EU limit for low pressure MSM is 100 mg/100 g (1000 ppm) calcium. MSM above this threshold is considered to be high pressure MSM.

TOR 1: Identify the public health risks linked to the different types of MSM and compare them as well with fresh meat, minced meat and meat preparations, as defined in EU legislation.

- Following consultation with the CONTAM experts, there is no increased risk from chemical hazards in MSM, compared to fresh meat, minced meat and meat preparations.

- Microbial hazards in pork and poultry MSM are expected to be similar to those in fresh meat, minced meat and meat preparations.

- Microbiological contamination of MSM depends on the hygiene of processing, the level and type of contamination in the raw material and its storage history.

- The risk of microbial growth increases with the degree of muscle fibre degradation and the associated release of nutrients. High pressure MSM may therefore provide a more favourable substrate for bacterial growth compared with low pressure MSM, hence the requirement that high pressure MSM be immediately frozen and only used in cooked products.

TOR 2: Identify and rank the parameters (e.g. muscle fibre modification, calcium content, water activity) to distinguish between these different types of MSM referred to in ToR 1 and compare them as well with fresh meat, minced meat and meat preparations, as defined in EU legislation;

- The following parameters were identified as potential indicators for the distinction of different types of MSM from non-MSM (fresh meat, minced meat and meat preparations):

  – Chemical parameters include calcium, phosphate, ash, iron, lipid (including cholesterol) and fatty acids (including the ones originating from bone marrow), moisture or water content, and protein (including collagen).

  – Histological parameters include microscopical detection of muscle, connective and adipose tissues, bone particles, cartilage, bone marrow and tissue from central nervous system, and their structural changes.

  – Molecular parameters could be also used including assays based on proteomics, metabolomics, electrophoretic techniques and immunological methods. However proper validation is still needed and in practice complexity and cost may limit their application.

  – Textural and rheological properties are not useful to discriminate different types of MSM from fresh meat, minced meat, and meat preparations because this analysis should be carried out on products with homogeneous structure rather than on particle-reduced products such as minced meat or low pressure MSM.

- In relation to ranking of the parameters in priority order, the following is concluded:
  - Analysis of available data, derived from published studies not specifically designed for this purpose, suggested that calcium content, which increases with pressure applied during processing, was the only appropriate chemical parameter that could be used to distinguish MSM from non-MSM products (fresh meat, minced meat, and meat preparations).
  - Low pressure MSM contains fewer bone particles than high pressure MSM and consequently lower calcium content. Therefore calcium content alone does not allow differentiation between low pressure MSM and other meat products.
  - Published data on cholesterol content, although limited, showed that this parameter could also be useful in the discrimination of MSM from non-MSM provided that additional data obtained by standardised methods confirm this observation.
  - Microscopic examination of tissue structure changes is a promising method for distinction between different types of MSM, minced meat and meat preparations, but further validation is needed because the available data do not provide objective threshold values.
  - Bone particles, detected microscopically, indicate the presence of MSM, but not all types of MSM contain bone particles. Therefore, they may not be used alone to consistently distinguish between MSM and non-MSM. The same is valid for cartilage particles.
  - For protein, ash and iron statistically significant differences were observed between MSM and non-MSM (fresh meat, minced meat and meat preparations); however, the discriminatory ability of the latter parameters was very low due to overlapping data. These parameters are affected to a large extent by raw material composition.
  - Other histological parameters related to tissue composition (muscle, connective tissue, adipose tissue, cartilage, bone marrow, central nervous tissue) do not provide clear differentiation between MSM and fresh meat, minced meat and meat preparations.

TOR 3: Establish the values for the parameters referred to in ToR 2

- The analysis of available published data suggested that the parameters of chemical composition of pork and poultry MSM that may be appropriate indicators for classifying a product as MSM are calcium and cholesterol content. Nevertheless the available data on cholesterol content in MSM are limited and do not support a definitive conclusion. Calcium content data analysed by species and animal body parts showed no major differences.
- A binary logistic model was developed in order to derive probability values for a product to be classified as hand deboned meat or MSM based on the calcium content. Calcium contents of 21, 39, 81.5 and 100 mg/100 g correspond to probabilities of 10%, 50%, 90% and 93.6% for a product to be classified as MSM (additional calcium contents and corresponding probabilities have been provided).
- The distinction of low pressure MSM from non-MSM products would need to be confirmed by the combination with other validated tests for parameters such as cholesterol content and microscopic detection of muscle fibre damage.
- The model behaviour was tested also with unpublished data provided by the meat industry and the results were consistent regarding hand deboned meat and high pressure MSM. However,

until specifically designed studies for validation become available, the outcome of this model cannot provide definitive conclusions on the differentiation between different types of MSM.

TOR 4: Propose objective methods (not subject to different interpretation) to measure the parameters referred to in ToR 2 and 3.

- The method specifically standardised for calcium determination in MSM (AOAC International method 983.19) is a simple titration method of the acid digested MSM using ethylene diamine tetra-acetate (EDTA). It is more usual for calcium to be determined by atomic absorption spectroscopy (AAS) but any method can be used, provided that it gives validated results.

RECOMMENDATIONS

- MSM production should be carried out under GHP/GMP and according to HACCP principles.

- Based on changes in processing and properties of derived MSM products, the classification and confirmatory testing of raw meat recovered after deboning should be also based on certain parameters of the final product, such as calcium content. New terminologies may be needed for low and high pressure MSM, because technological advances have resulted in low pressure products resembling minced meat. For example "low pressure MSM" could be simply called "mechanically deboned meat (MDM)", while "high pressure MSM" could be named "high calcium mechanically separated meat (HCaMSM)".

- Specifically designed studies for the collection of data obtained by standardised methods on potential indicators, especially calcium and cholesterol, should be undertaken as this could lead to an improved method for MSM identification. Additional analysis in these studies could include histological examination.

- Studies on differentiation of MSM from other meat products based on the analysis of combination of different parameters (chemical, physical, etc.) should also be undertaken.

DOCUMENTATION PROVIDED TO EFSA

1. Groves K. 2011. Evaluation of a simple microscopy protocol for identifying mechanically separated meat (MSM) in pork, chicken and turkey. Submitted by Leatherhead Food Research.

2. Groves K. 2011. Standard operating procedure (SOP) 001. Standard operating procedure for a microscopy protocol for identifying mechanically separated meat (MSM) from pork, turkey and chicken. Submitted by Leatherhead Food Research.

3. Coton J.P. Matière première de viande – Spécifications et méthode d'essai. Rapport de l'etude de faisabilite d'un accord europeen. Submitted by Histalim, Montpellier, France.

4. McNitt J.I., Negatu Z., McMillin K. Bone particle determination in mechanically separated rabbit meat- preliminary results. Proceedings - 8th World Rabbit Congress – September 7-10, 2004 – Puebla, Mexico.

5. Summary of microbiological and chemical analysis of mechanically separated meats (MSM) and mechanically separated poultry meats (MSPM) performed in NVRI in Pulawy. Selected problems. This refers to the following publications:

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APPENDIX

A. DATABASE ON CHEMICAL CHARACTERISTICS OF HAND DEBONED, MINCED MEAT AND MSM MEAT FROM POULTRY MEAT AND PORK DEVELOPED FROM THE LITERATURE DATA.

Method	Mechanic method	Animal	Animal part	Protein %	Ash %	Calcium mg/100 g	Iron mg/100 g	Cholesterol mg/100 g	Reference
Mechanically	Pressure (40 lb/in^2)	Poultry	-	20.65	1.05	58.2	1		Barbut at al., 1989
Mechanically	Pressure (75 lb/in^2)	Poultry	-	20.76	1.04	53.4	1.17		Barbut at al., 1989
Mechanically	Pressure (120 lb/in^2)	Poultry	-	20.1	1.12	56.8	1.06		Barbut at al., 1989
Mechanically	Pressure (150 lb/in^2)	Poultry	-	20.68	1.23	76.4	1.785		Barbut at al., 1989
Hand-deboned	-	Poultry	-	23.67	0.94	16.4	0.625		Barbut at al., 1989
Mechanically	Beehive	Poultry (chicken)	Carcass	15.57	0.406	60			Botka-Petrak et al., 2011
Mechanically	Beehive	Poultry (chicken)	Back	13.46	1.18	195			Botka-Petrak et al., 2011
Mechanically	Beehive	Poultry (chicken)	Wings	14.564	1.656	293.6			Botka-Petrak et al., 2011
Mechanically	Beehive	Poultry (chicken)	Neck (deboned meat)	14.892	1.37	216			Botka-Petrak et al., 2011
Mechanically	Beehive	Poultry (chicken)	Carcasses (offal products)	18.52	12.66	6436			Botka-Petrak et al., 2011
Mechanically	Beehive	Poultry (chicken)	Back (offal products)	23.88	20.196	7410			Botka-Petrak et al., 2011
Mechanically	Beehive	Poultry (chicken)	Wings (offal products)	18.32	9.458	3368			Botka-Petrak et al., 2011
Mechanically	Beehive	Poultry (chicken)	Neck (offal products)	21.13	16.88	7074			Botka-Petrak et al., 2011
Hand-deboned	-	Pork	-	18.04	0.92	6.12	1.2	62.33	Calhoun et al., 1999
Mechanically	Pressure (160-180 bar for 2sec)	Pork	Backbone, neckbone, aitch bone, hip bone, scapula	15.38	1.14	107.5	3.24	101.67	Calhoun et al., 1999
Hand-deboned	-	Pork	Backbone, neckbone, aitch bone, hip bone, scapula	17.24	0.93	26.77	1.11	72.33	Calhoun et al., 1999
Mechanically	-	Pork	Shoulder blade		1.1	70.2	3.7		(Crosland et al., 1995)
Hand-deboned	-	Pork	Shoulder blade		1	55.4	1.5		Crosland et al., 1995
Mechanically	-	Pork	Chine		1.6	206.2	3.8		Crosland et al., 1995
Hand-deboned	-	Pork	Chine		1.3	241.9	0.5		Crosland et al., 1995
Mechanically	-	Poultry (chicken)	Back		1	84.1	1.6		Crosland et al., 1995
Hand-deboned	-	Poultry (chicken)	Back		0.8	53.8	0.9		Crosland et al., 1995
Mechanically	-	Poultry (turkey)	Large frames		0.9	43.6	1.5		Crosland et al., 1995
Hand-deboned	-	Poultry (turkey)	Large frames		0.8	19.6	1.6		Crosland et al., 1995
Mechanically	Protecon (Pressure 235 bar for 4sec)	Pork	-		1.6	206.2	3.83		Crosland et al., 1995
Mechanically	Protecon (Pressure 215 bar for 4sec)	Pork	-		1.7	213.8	3.95		Crosland et al., 1995
Mechanically	Protecon (Pressure 215 bar for 8sec)	Pork	-		1.5	155	3.92		Crosland et al., 1995

Method	Mechanic method	Animal	Animal part	Protein %	Ash %	Calcium mg/100 g	Iron mg/100 g	Cholesterol mg/100 g	Reference
Mechanically	Protecon (Pressure 235 bar for 8sec)	Pork	-		1.2	81.8	3.99		Crosland et al., 1995
Mechanically	Protecon (Pressure 280 bar for 6sec)	Poultry (chicken)	Back		0.8	65.4	1.7		Crosland et al., 1995
Mechanically	Protecon (Pressure 250 bar for 6sec)	Poultry (chicken)	Back		0.8	62.9	1.7		Crosland et al., 1995
Mechanically	Yieldmaster (setting 6 on dial)	Poultry (chicken)	Back		1.3	239.8	2.1		Crosland et al., 1995
Mechanically	Yieldmaster (setting 3 on dial)	Poultry (chicken)	Back		1.3	191.5	2		Crosland et al., 1995
Mechanically	Protecon (Pressure 280 bar for 6sec)	Poultry (turkey)	Large frames		0.78	35.5	1.4		Crosland et al., 1995
Mechanically	Protecon (Pressure 280 bar for 4sec)	Poultry (turkey)	Large frames		0.89	43.6	1.5		Crosland et al., 1995
Mechanically	Protecon (Pressure 250 bar for 6sec)	Poultry (turkey)	Large frames		0.85	34.2	1.4		Crosland et al., 1995
Mechanically	Protecon (Pressure 250 bar for 4sec)	Poultry (turkey)	Large frames		0.79	35.6	1.5		Crosland et al., 1995
Mechanically	Yieldmaster (setting 7 on dial)	Poultry (turkey)	Large frames		1.36	423.8	1.1		Crosland et al., 1995
Mechanically	Yieldmaster (setting 9 on dial)	Poultry (turkey)	Large frames		1.26	225	1.4		Crosland et al., 1995
Mechanically	Yieldmaster (setting 10 on dial)	Poultry (turkey)	Large frames		1.39	516.2	1.1		Crosland et al., 1995
Mechanically	Yieldmaster (setting 11 on dial)	Poultry (turkey)	Large frames		1.18	442	1.1		Crosland et al., 1995
Mechanically	-	Poultry (chicken)	Racks and marrow		2.2	1429	2.7		Crosland et al., 1995
Mechanically	-	Poultry (chicken)	Wings		1.6	488	1.9		Crosland et al., 1995
Mechanically	-	Pork	Shoulder blade, forelimb and marrow)		1	63.7	4.2		Crosland et al., 1995
Mechanically	-	Pork	Neck and rib		1	64	3.3		Crosland et al., 1995
Mechanically		Poultry (chicken)	-	10.58	1.17				(Daros et al., 2005)
Hand-deboned	-	Poultry	Breast	23.1	1	11	0.7	58	Field, 2004
Hand-deboned	-	Poultry	Leg	20.1	0.9	11	1	80	Field, 2004
Mechanically	-	Poultry	Back and neck	11.4	1	118	1.6	140	Field, 2004
Mechanically	-	Poultry	Back and neck	13.8	1	133	1.7	120	Field, 2004
Mechanically	-	Poultry	Mature hens	20.4	1.3	112	1.3	122	Field, 2004
Mechanically	-	Poultry	Mature hens	20.4	1.3	130	1.3	110	Field, 2004
Hand-deboned	-	Pork	-	17	0.9	5	0.8	74	Field, 2004
Mechanically	-	Pork	-	15.4	1.1	106	3	126	Field, 2004
Mechanically	Beehive	Pork	Ham	10.21	4.07	1390			(Field et al., 1976)

Method	Mechanic method	Animal	Animal part	Protein %	Ash %	Calcium mg/100 g	Iron mg/100 g	Cholesterol mg/100 g	Reference
Mechanically	Beehive	Pork	Picnic	9.06	3.68	1220			Field, 1976
Mechanically	Beehive	Pork	Boston butt	13.5	2.71	730			Field, 1976
Mechanically	Beehive	Pork	Loin	14.01	1.77	410			Field, 1976
Hand-deboned	-	Pork	Ham	15.67	0.54	29			Field, 1976
Hand-deboned	-	Pork	Picnic	19.17	0.68	43			Field, 1976
Hand-deboned	-	Pork	Boston butt	19.21	0.86	79			Field, 1976
Hand-deboned	-	Pork	Loin	16.72	0.72	37			Field, 1976
Mechanically	-	Poultry (chicken)	Back and neck	11.98	0.77	107			Froning et al., 1981
Mechanically	-	Poultry (fowl, cooked)	-	18.28	1.38				Froning et al., 1981
Mechanically	-	Poultry (turkey)	Frame	16.28	1.25				Froning et al., 1981
Mechanically	-	Poultry (chicken, unwashed)	-	4.66					Froning and McKee, 2001
Mechanically	-	Poultry (chicken, washed with tap water)	-	7.42					Froning and McKee, 2001
Mechanically	-	Poultry (chicken, washed with 0.1M NaCl)	-	7.48					Froning and McKee, 2001
Mechanically	-	Poultry (chicken, washed with sodium phosphate buffer)	-	7.01					Froning and McKee, 2001
Mechanically	-	Poultry (chicken, washed with 0.5% $NaHCO_3$)	-	7.12					Froning and McKee, 2001
Mechanically	-	Poultry (chicken, unwashed, cooked)	-	4.56					Froning and McKee, 2001
Mechanically	-	Poultry (chicken, washed with tap water, cooked)	-	5.94					Froning and McKee, 2001
Mechanically	-	Poultry (chicken, washed with 0.1M NaCl, cooked)	-	6.86					Froning and McKee, 2001
Mechanically	-	Poultry (chicken, washed with sodium phosphate buffer, cooked)	-	6.98					Froning and McKee, 2001
Mechanically	-	Poultry (chicken, washed with 0.5% $NaHCO_3$, cooked)	-	6.79					Froning and McKee, 2001
Mechanically	-	Poultry (chicken)	Back	13.2					Froning and McKee, 2001
Mechanically	-	Poultry	Neck	15.3					Froning and McKee, 2001

Method	Mechanic method	Animal	Animal part	Protein %	Ash %	Calcium mg/100 g	Iron mg/100 g	Cholesterol mg/100 g	Reference
Mechanically	-	Poultry (cooked)	Spent layer	18.3					Froning and McKee, 2001
Mechanically	Beehive	Poultry (turkey)	Carcass						(Mielnik et al., 2003)
Mechanically	Beehive	Poultry (chicken)	Dorsal part, neck	11	0.7				(Negrão et al., 2005)
Mechanically	Beehive	Poultry (chicken)	Dorsal part, neck	84.3	5.7				Negrão et al., 2005
Hand-deboned	-	Poultry (chicken)	Breast	24	1.12				Negrão et al., 2005
Hand-deboned	-	Poultry (chicken)	Breast	77.3	4				Negrão et al., 2005
Mechanically	-	Pork	Neck	14.7	1.5	350			Newman, 1981
Mechanically	-	Pork	Rib	15.5	1.2	200			Newman, 1981
Mechanically	-	Pork	Mixture	12.5	1.4	300			Newman, 1981
Mechanically	-	Poultry (chicken)	Back and neck	12.3					Newman, 1981
Mechanically	-	Poultry (chicken)	Spent layer	14.1					Newman, 1981
Mechanically	-	Poultry (chicken)	Broiler neck	12.4	0.7	40			Newman, 1981
Hand-deboned	-	Poultry (chicken)	Broiler neck	12	0.5	10			Newman, 1981
Mechanically	-	Poultry (turkey)	Frame	12.8					Newman, 1981
Mechanically	Machine with continuous action separating ground bones through a strainer under pressure	Pork	Back			450			Newman, 1981
Mechanically	Machine with continuous action separating ground bones through a strainer under pressure	Pork	Neck			400			Newman, 1981
Mechanically	Machine with continuous action separating ground bones through a strainer under pressure	Pork	Ham			1650			Newman, 1981
Mechanically	Machine with continuous action separating ground bones through a strainer under pressure	Pork	Picnic			1810			Newman, 1981
Mechanically	Machine with batch action using crushed bones forced against a stationary strainer	Pork	Back			150			Newman, 1981
Mechanically	Machine with batch action using crushed bones forced against a stationary strainer	Pork	Neck			150			Newman, 1981

Method	Mechanic method	Animal	Animal part	Protein %	Ash %	Calcium mg/100 g	Iron mg/100 g	Cholesterol mg/100 g	Reference
Mechanically	Machine with batch action using crushed bones forced against a stationary strainer	Pork	Ham			160			Newman, 1981
Mechanically	Machine with batch action using crushed bones forced against a stationary strainer	Pork	Picnic			170			Newman, 1981
Mechanically	Machine with continuous action separating ground bones using a stationary strainer	Pork	Back			730			Newman, 1981
Mechanically	Machine with continuous action separating ground bones using a stationary strainer	Pork	Neck			550			Newman, 1981
Mechanically	Machine with continuous action separating ground bones using a stationary strainer	Pork	Ham			1520			Newman, 1981
Mechanically	Machine with continuous action separating ground bones using a stationary strainer	Pork	Picnic			1440			Newman, 1981
Mechanically	Machine with continuous action separating ground bones through a strainer under pressure	Pork	Back			460			Newman, 1981
Mechanically	Machine with continuous action separating ground bones through a strainer under pressure	Pork	Neck			230			Newman, 1981
Mechanically	Machine with continuous action separating ground bones through a strainer under pressure	Pork	Ham						Newman, 1981

Method	Mechanic method	Animal	Animal part	Protein %	Ash %	Calcium mg/100 g	Iron mg/100 g	Cholesterol mg/100 g	Reference
Mechanically	Machine with continuous action separating ground bones through a strainer under pressure	Pork	Picnic						Newman, 1981
Mechanically	-	Pork	Boneless loin meat and desinewed	22.53	1.01				(Osburn et al., 1995)
Mechanically	-	Pork	Boneless loin meat and desinewed	31.1	1.2				Osburn et al., 1995
Hand-deboned	-	Poultry (chicken)	Breast	24.6					(Perlo et al., 2006)
Mechanically	-	Poultry (chicken)	-	13.6					Perlo et al., 2006
Mechanically	-	Poultry (chicken)	-	9.4					Perlo et al., 2006
Hand-deboned	Manual trimming and coventional mincer	Poultry (turkey)	Drumstick, wing and broiler thigh (boneless)	18.9					(Petracci et al., 2012)
Mechanically	-	Poultry (turkey)	Drumstick, wing and broiler thigh (boneless)	17.9					Petracci et al., 2012
Mechanically	Protecon machine - normal pressure	Pork	Neck	14.6	1	80	3.5		(Savage et al., 1995)
Mechanically	Protecon machine - high pressure	Pork	Neck	14.8	1	77	3.5		Savage et al., 1995
Mechanically	Protecon machine - high pressure	Pork	Narrow bone	14.6	1	80	3		Savage et al., 1995
Mechanically	Protecon machine - normal pressure	Poultry (chicken)	Frame	15.4	1	58.5	2		Savage et al., 1995
Mechanically	Protecon machine - normal pressure	Poultry (chicken)	Frame	17.9	1.2	89	2		Savage et al., 1995
Mechanically	Protecon machine - high pressure	Poultry (chicken)	Frame	15.6	1	76.5	2		Savage et al., 1995
Mechanically	Protecon machine - high pressure	Poultry (chicken)	Frame	18.1	1	55	2		Savage et al., 1995
Mechanically	Protecon machine - normal pressure	Poultry (chicken)	Front end	15.7	1	64.5	1.5		Savage et al., 1995
Mechanically	Protecon machine - high pressure	Poultry (chicken)	Front end	16.1	1	61.5	3		Savage et al., 1995
Mechanically	Protecon machine - normal pressure	Poultry (chicken)	Back	13.8	0.9	34.5	2.5		Savage et al., 1995
Mechanically	Protecon machine - high pressure	Poultry (chicken)	Back	14.3	1	66	2		Savage et al., 1995
Mechanically	Protecon machine - high pressure	Poultry (chicken)	Front end and back	14.5	1	76.5	2.5		Savage et al., 1995
Mechanically	Baader machine-1.3mm drum	Poultry (chicken)	Frame	1	1	57	2.5		Savage et al., 1995
Mechanically	Baader machine-1.3mm drum	Poultry (chicken)	Neck (cooked)	22.6	1.1	121	2		Savage et al., 1995

Method	Mechanic method	Animal	Animal part	Protein %	Ash %	Calcium mg/100 g	Iron mg/100 g	Cholesterol mg/100 g	Reference
Mechanically	Baader machine-2mm drum	Poultry (chicken)	Back	14	0.9	57.5	2		Savage et al., 1995
Mechanically	Baader machine-2mm drum	Poultry (chicken)	Carcass (cooked)	20.2	1.1	136.5	2		Savage et al., 1995
Mechanically	Baader machine-3mm drum	Poultry (chicken)	Carcass (cooked)	21.1	1.1	199	2		Savage et al., 1995
Mechanically	-	Poultry (chicken)	Commercial sample cooked	17.3	0.8	144.5	1.5		Savage et al., 1995
Mechanically	Yieldmaster machine - standard	Poultry (chicken)	Standard production material	14.8	1	70.5	3		Savage et al., 1995
Mechanically	Yieldmaster machine - high	Poultry (chicken)	Standard production material and parson's nose	14.9	1	73	2		Savage et al., 1995
Mechanically	Poss machine	Poultry (chicken)	Standard production material	17.4	1.3	146.5	2		Savage et al., 1995
Mechanically	Poss machine	Poultry (chicken)	Standard production material and parson's nose and low fat	12.8	1	86	1.5		Savage et al., 1995
Mechanically	Poss machine	Poultry (chicken)	Standard production material and parson's nose and high fat	13.8	1	99	2		Savage et al., 1995
Mechanically	Protecon	Poultry (turkey)	-	13.8	0.8	54	1.3		Savage et al., 1995
Hand-deboned	Minced	Pork	Shoulder, rind off excluding knuckle	18.8	1	7	1		Savage et al., 1995
Hand-deboned	Minced/colloid milled	Pork	Shoulder, rind off excluding knuckle	15	0.8	0.5	1.1		Savage et al., 1995
Hand-deboned	Minced	Pork	Headmeat, 90% visual lean	17	0.9	25	2.4		Savage et al., 1995
Hand-deboned	Minced	Pork	Headmeat, 50% visual lean	14.9	0.6	8	1.2		Savage et al., 1995
Hand-deboned	Hand trimmed from the bones/minced	Pork	Bones	19.2	1	40	1.8		Savage et al., 1995
Hand-deboned	Bone removed with Protecon Auto Deboner machine/minced *	Pork	Shoulder	20.7	1	5.5	1.5		Savage et al., 1995
Hand-deboned	Minced	Poultry (chicken)	Light meat-breast skin	21.3	1	8.5	1		Savage et al., 1995
Hand-deboned	Minced/colloid milled	Poultry (chicken)	Light meat-breast skin	18.5	0.9	7	0.5		Savage et al., 1995
Hand-deboned	Minced	Poultry (chicken)	Dark meat-thigh	19.4	0.9	6.5	1		Savage et al., 1995
Hand-deboned	Minced/colloid milled	Poultry (chicken)	Dark meat-thigh	15.8	0.8	7.5	1		Savage et al., 1995
Hand-deboned	Bone removed with Protecon Auto Deboner machine/put through a Baader mechanical seperation machine/minced*	Poultry (turkey)	Neck	19.6	1	25	2.5		Savage et al., 1995

Method	Mechanic method	Animal	Animal part	Protein %	Ash %	Calcium mg/100 g	Iron mg/100 g	Cholesterol mg/100 g	Reference
Hand-deboned	Bone removed with Protecon Auto Deboner machine/minced*	Poultry (turkey)	Neck	19.5	1	14	2.5		Savage et al., 1995
Hand-deboned	Bone removed with Protecon Auto Deboner machine/minced*	Poultry (turkey)	Drumstick	19.3	0.9	25	1.6		Savage et al., 1995
Hand-deboned	Bone removed with Protecon Auto Deboner machine/minced*	Poultry (turkey)	Drumstick	19.8	0.9	10.5	2		Savage et al., 1995
Hand-deboned	Debonding with sharp knives and after two times in meat grinder	Poultry (turkey)	Carcass	20.1	1	1.72	1.35	56.9	(Serdaroglu et al., 2005)
Mechanically	Smooth Deboner Machine	Poultry (turkey)	Carcass	15.5	0.9	20.3	1.3	63.6	Serdaroglu et al., 2005
Mechanically	RM 500 machine	Poultry (chicken)	Dorsal part	12.2	0.4				(Stangierski et al., 2008)
Mechanically	-	Poultry (chicken)	-	15.5	1.3				Trindade et al., 2004
Mechanically	-	Poultry (chicken)	-	15.4	1.2				Trindade et al., 2004
Mechanically	-	Poultry (chicken)	-	14.2					Trindade et al., 2004
Mechanically	-	Poultry (chicken)	-	13.9					Trindade et al., 2004
Mechanically	-	Poultry (chicken)	Back	8.5	0.6				Trindade et al., 2004
Mechanically	-	Poultry (chicken)	Back	12.4	1.1				Trindade et al., 2004
Mechanically	-	Poultry (chicken)	Back and neck	9.3					Trindade et al., 2004
Mechanically	-	Poultry (chicken)	Back and neck	13.4					Trindade et al., 2004
Hand-deboned	-	Poultry (chicken)	-	23.1	1.2				Trindade et al., 2004
Hand-deboned	-	Poultry (chicken)	-	19.5	1				Trindade et al., 2004
Mechanically	-	Poultry	-		1.08				(Yuste et al., 1999)
Mechanically	-	Pork	-		0.92				Yuste et al., 1999
Mechanically	-	Poultry	Meat remain on carcasses and left overs		0.94				Yuste et al., 2002
Mechanically	-	Poultry (turkey)	Neck	17.2					Viuda-Martos et al. 2012
Mechanically	-	Poultry (turkey)	-	13.2					Viuda-Martos et al. 2012
Mechanically	-	Poultry (chicken)	Carcass	14.72					Viuda-Martos et al. 2012
Mechanically	-	Pork	Ham	11.52					Viuda-Martos et al. 2012
Mechanically	-	Poultry (turkey)	-						Viuda-Martos et al. 2012
Mechanically	-	Poultry (chicken)	Half frames	13.93	1.16				(Rivera et al., 2000)
Hand-deboned	-	Pork	Leg			26			Branscheid and Judas, 2009
Mechanically	-	Pork	Leg			121			Branscheid and Judas, 2009

Method	Mechanic method	Animal	Animal part	Protein %	Ash %	Calcium mg/100 g	Iron mg/100 g	Cholesterol mg/100 g	Reference
Hand-deboned	-	Pork	Head			78			Branscheid and Judas, 2009
Mechanically	-	Pork	Head			183			Branscheid and Judas, 2009
Hand-deboned	-	Pork	Shoulder			27			Branscheid and Judas, 2009
Mechanically	-	Pork	Shoulder			152			Branscheid and Judas, 2009
Hand-deboned	-	Poultry (chicken)	Breast			18			Branscheid and Judas, 2009
Mechanically	-	Poultry (chicken)	Breast			113			Branscheid and Judas, 2009
Hand-deboned	-	Poultry (chicken)	Neck			28			Branscheid and Judas, 2009
Mechanically	-	Poultry (chicken)	Neck			139			Branscheid and Judas, 2009
Hand-deboned	Minced	Poultry (chicken)	Carcass	20.85	0.98	16.75	5.3	78.7	(Al-Najdawi and Abdullah, 2002)
Hand-deboned	Minced	Poultry (chicken)	Carcass	22.65	1.1	13.5	4.6	34.29	Al-Najdawi and Abdullaha (2002)
Mechanically	Protecon	Poultry (chicken)	Carcass	20.45	1.25	162.5	5.5	122.55	Al-Najdawi and Abdullaha (2002)
Mechanically	Protecon	Poultry (chicken)	Carcass	20.35	0.32	230	4.2	58.75	Al-Najdawi and Abdullaha (2002)
Hand-deboned	-	Poultry (turkey)	Wings					46	(Baggio et al., 2002)
Hand-deboned	-	Poultry (turkey)	Leg					35	Baggio et al. (2002)
Hand-deboned	-	Poultry (turkey)	Breast					27	Baggio et al. (2002)
Hand-deboned	-	Poultry (chicken)	Carcass	20		13	1.1	110	(Barroeta, 2007)
Hand-deboned	-	Poultry (chicken)	Breast	21.8		14	1	69	Barroeta (2007)
Hand-deboned	-	Poultry (chicken)	Leg	18.83				80.3	(Almeida et al., 2006)
Hand-deboned	-	Pork	Loin					57	(Dorado et al., 1999)
Hand-deboned		Pork	Tenderloin					72	Dorado et al. (1999)
Hand-deboned		Pork	Spare ribs					73	Dorado et al. (1999)
Hand-deboned		Pork	Leg					60	Dorado et al. (1999)
Hand-deboned		Pork	Hind-cock					67	Dorado et al. (1999)
Hand-deboned		Pork	Longissimus dorsi	22.7				46.1	Hernandez et al. (1998)
Hand-deboned		Pork	Biceps femoris	21.9				52.2	Hernandez et al. (1998)
Hand-deboned		Pork	Triceps brachii	21.4				51.3	(Hernandez et al., 1998)
Hand-deboned		Pork	Biceps femoris				1.3	54	(Costa et al., 2009)
Hand-deboned		Pork	Longissimus dorsi				1.29	50	Costa et al. (2009)
Hand-deboned		Pork	Supra spinatus				1.57	54	Costa et al. (2009)
Hand-deboned		Pork	Chop					46.9	Piironen et al. (2005)
Hand-deboned		Pork	Longissimus dorsi					45	Piironen et al. (2005)
Hand-deboned		Poultry (chicken)	Breast					56.2	Piironen et al. (2005)
Hand-deboned		Poultry (chicken)	Leg					84	(Piironen et al., 2002)
Hand-deboned		Pork	Longissimus dorsi					47	(Sinclair et al., 2010)
Hand-deboned		Pork	Mince					54	Sinclair et al., (2010)
Hand-deboned		Poultry (chicken)	Breast					53	(Komprda et al., 2003)

Method	Mechanic method	Animal	Animal part	Protein %	Ash %	Calcium mg/100 g	Iron mg/100 g	Cholesterol mg/100 g	Reference
Hand-deboned		Poultry (chicken)	Thigh					82.9	Komprda et al. (2003)
Hand-deboned		Poultry (turkey)	Breast					53	Komprda et al. (2003)
Hand-deboned		Poultry (turkey)	Thigh					61.5	Komprda et al. (2003)
Hand-deboned		Poultry (chicken)	Breast					47.11	Ponte et al. (2008)
Hand-deboned		Poultry (chicken)	Breast					59.3	(Rule et al., 2002)
Hand-deboned		Poultry (turkey)	Breast	24.38	1.43	16.11	4.526		(Karakök et al., 2010)
Hand-deboned		Poultry (chicken)	Breast	22.33	1	7.83	4.175		(Karakök et al., 2010)
Mechanically	Canadian type	Poultry (chicken)	Neck	12.6	1.3	120	1.8		(Hamm and Searcy, 1981)
Mechanically	Yieldmaster	Poultry (chicken)	Neck	12.5	1	158	1.7		Hamm and Searcy (1981)
Mechanically	Canadian type	Poultry (chicken)	Neck	11.9	1	98	1.3		Hamm and Searcy (1981)
Mechanically	Canadian type	Poultry (chicken)	Breast and rib bones	14	1.4	152	2		Hamm and Searcy (1981)
Mechanically	Yieldmaster	Poultry (chicken)	Breast and rib bones	15.5	1.5	255	2.7		Hamm and Searcy (1981)
Mechanically	Beehive	Poultry (chicken)	Frame	15.5	1.3	202	1.4		Hamm and Searcy (1981)
Mechanically	Yieldmaster	Poultry (turkey)	Frame	13.5	1.2	221	1.4		Hamm and Searcy (1981)
Mechanically	Beehive	Poultry (chicken)	Neck	15.3		94.7	1.5		Hamm and Searcy (1981)
Mechanically		Poultry (chicken)	Frame			108	3		Hamm and Searcy (1981)
Mechanically		Poultry (chicken)	Frame			213			Hamm and Searcy (1981)
Mechanically		Poultry (chicken)		14.7	1.2	187	1.2		Hamm and Searcy (1981)
Mechanically		Poultry (turkey)	Racks	15.4		153	7.5		Hamm and Searcy (1981)
Mechanically		Poultry (turkey)	Frame			143	4.3		Hamm and Searcy (1981)
Mechanically		Poultry (turkey)	Frame	13.3	1.1	145	1.6		Hamm and Searcy (1981)
Mechanically	Protecon	Poultry (chicken)				194			(Lyon et al., 1978)
Mechanically	Baader	Poultry (chicken)				105			(Nagy et al., 2007)
Hand-deboned		Poultry (chicken)	Breast			61			Nagy et al., (2007)
Hand-deboned		Poultry (chicken)	Thigh			58			Nagy et al., (2007)
Hand-deboned		Poultry (chicken)	Breast			29			(Suchy et al., 2002)
Hand-deboned		Poultry (chicken)	Thigh			30			Suchy et al. (2002)
Hand-deboned		Poultry (chicken)	Breast			29			Suchy et al. (2002)
Hand-deboned		Poultry (chicken)	Thigh			31			Suchy et al. (2002)
Hand-deboned		Poultry (chicken)	Breast			29			Suchy et al. (2002)
Hand-deboned		Poultry (chicken)	Thigh			31			Suchy et al. (2002)
Hand-deboned		Poultry (turkey)	Breast					54	(Wong et al., 1993)
Hand-deboned		Poultry (turkey)	Thigh					84	Wong et al. (1993)
Mechanically	Poss machine	Poultry (chicken)	Back	12.77	0.92	52.07	1.61	168.89	(Kolsarici et al., 2010)
Mechanically	Poss machine	Poultry (chicken)	Breast	16.9	1.45	179.52	3.37	82.74	Kolsarici, et al., 2010
Mechanically	Poss machine	Poultry (chicken)	Neck	12.27	0.88	56.8	2.06	82.34	Kolsarici, et al., 2010

Method	Mechanic method	Animal	Animal part	Protein %	Ash %	Calcium mg/100 g	Iron mg/100 g	Cholesterol mg/100 g	Reference
Hand-deboned	-	Poultry (chicken)	-						(Püssa et al., 2009)
Hand-deboned	-	Poultry (turkey)	-						Pussa et al., 2009
Hand-deboned	-	Pork	-						Pussa et al., 2009
Mechanically	Beehive	Poultry (chicken)	-						Pussa et al., 2009
Mechanically	-	Poultry (turkey)	-						Pussa et al., 2009
Mechanically	-	Pork	-						Pussa et al., 2009
Mechanically	-	Poultry (chicken)	-	32.56	2.15				Rossi et al., 2009
Mechanically	Beehive	Poultry (chicken)	Frame	12.55	0.93				(Özkeçeci RB et al., 2008)
Mechanically	Beehive	Poultry (chicken)	Neck	12.4	0.93				(Özkeçeci RB et al., 2008)
Mechanically	-	Poultry (chicken)	Back	14.27		35.5	1.287		Henckel et al., 2004
Mechanically	Baader	Poultry (chicken)	Back	13.98		25.2	1.657		Henckel et al., 2004
Hand-deboned	-	Poultry (chicken)	Back	14.76		16.4	1.011		Henckel et al., 2004
Mechanically	-	Poultry (chicken)	Breast	18.1		122.3	2.243		Henckel et al., 2004
Mechanically	Baader	Poultry (chicken)	Breast	18.78		33.6	1.879		Henckel et al., 2004
Hand-deboned	-	Poultry (chicken)	Breast	20.14		31.4	1.663		Henckel et al., 2004
Mechanically	Protecon	Pork	Ribs and backs	16.9	1.24	130			Koolmees et al., 1986
Mechanically	Protecon	Pork	Ham and shoulder	13.6	1.04	60			Koolmees et al., 1986
Mechanically	Protecon	Pork	Sow's bones	15.2	1.27	190			Koolmees et al., 1986
Mechanically	Protecon	Pork	Porker's bones	14.3	1.41	280			Koolmees et al., 1986
Mechanically	Protecon	Pork	Ham	15.6	1.48	110			Koolmees et al., 1986
Mechanically	Protecon	Pork	Shoulder	12.6	1	70			Koolmees et al., 1986
Mechanically	Protecon	Pork	Head	14.9	0.94	60			Koolmees et al., 1986
Mechanically	Protecon	Poultry (chicken)	Carcasses and backs	15.5	1.14	110			Koolmees et al., 1986
Mechanically	Paoli	Poultry (chicken)	Carcasses and backs	14.9	1.45	220			Koolmees et al., 1986
Mechanically	Beehive	Poultry (chicken)	Carcasses, backs, necks and wings	17.7	1.62	250			Koolmees et al., 1986
Mechanically	Protecon	Poultry (chicken)	Carcasses, backs, necks and wings	14.6	0.99	70			Koolmees et al., 1986
Mechanically	Beehive	Poultry (chicken)	Carcass	14.4	1.11	150			Koolmees et al., 1986
Mechanically	Protecon	Poultry (chicken)	Carcass	17.4	1.25	130			Koolmees et al., 1986
Mechanically	Hydraulic pressure machine	Poultry (chicken)	-	38.1					(Perlo et al., 2003)
Mechanically	Lima	Poultry (turkey)	Dorsal part	15.8	1				(Stangierski and Kijowski, 2003)
Mechanically	Hollow drum type	Poultry (chicken)	Dorsal part	16.655	1.135	55	22.315		(Gonçalves et al., 2009)
Mechanically	Hollow drum type	Poultry (chicken)	Dorsal part	16.87	1.225	70	22.6		Golcanves et al., 2009
Mechanically	-	Poultry (chicken)	Back	10.8		53		94.6	(Ang and Hamm, 1982)
Mechanically	-	Poultry (chicken)	Neck	11.5		91		94.2	Ang and Hamm (1982)

Method	Mechanic method	Animal	Animal part	Protein %	Ash %	Calcium mg/100 g	Iron mg/100 g	Cholesterol mg/100 g	Reference
Mechanically	-	Poultry (chicken)	Neck	10.3		91		109.4	Ang and Hamm (1982)
Mechanically	-	Poultry (chicken)	Upper backs	11.9				129.1	Ang and Hamm (1982)
Hand-deboned	-	Poultry (chicken)	Back	12.95		48		81	Ang and Hamm (1982)
Hand-deboned	-	Poultry (chicken)	Neck	13.38		35		75	Ang and Hamm (1982)
Hand-deboned	-	Poultry (chicken)	Neck	13.87		60		98	Ang and Hamm (1982)
Hand-deboned	-	Poultry (chicken)	Neck	9.96		50		94	Ang and Hamm (1982)
Mechanically	Beehive AU 4171	Poultry (turkey)		17		54.3	1.9		(Allred et al., 1990)
Hand-deboned		Poultry (turkey)		16.9		6	1.1		Allred et al. (1990)
Mechanically	Jack Prince	Poultry	-			110			(Germs and Steunenberg, 1978)
Mechanically	Bibun	Poultry	-			380			Germs and Steunenberg (1978)
Mechanically	Bibun	Poultry	-			210			Germs and Steunenberg (1978)
Mechanically	Paoli	Poultry	-			150			Germs and Steunenberg (1978)
Mechanically	Bibun	Poultry	-			240			Germs and Steunenberg (1978)
Mechanically	Beehive	Poultry	-			200			Germs and Steunenberg (1978)
Mechanically		Poultry (chicken)	Whole	15.39	1.19	190	1.2		Mott et al. (1982)
Mechanically		Poultry (chicken)	Frame	16.19	1.28	200	4.25		Mott et al. (1982)
Mechanically		Poultry (chicken)	Frame	16.55	1.39	230	4.05		Mott et al. (1982)
Mechanically	Protecon	Poultry (chicken)	Back and neck	15	1	120			Mast et al. (1982)
Mechanically	Beehive	Poultry (chicken)	Back and neck	13.3	1.3	200			Mast et al. (1982)
Mechanically	Paoli	Poultry (chicken)	Back and neck	13.8	1.6	350			Mast et al. (1982)
Mechanically	Yieldmaster	Poultry (chicken)	Back and neck	13.8	1	110			Mast et al. (1982)
Mechanically	Poss machine	Poultry (chicken)	Carcass minus breast	13.6	1.4	299	2.4	61	(Contreras-Castillo et al., 2008)
Mechanically	Poss machine	Poultry (chicken)	Carcass minus breast	15.2	2.1	448	2.3	73	Contreras-Castillo et al. (2008)
Mechanically	Protecon	Pork	Ribs and backs	16.9		130			Bijker et al. (1983)
Mechanically	Protecon	Pork	Bones	15.2		190			Bijker et al. (1983)
Mechanically	Protecon	Pork	Mixture (porkers)	15.8		120			Bijker et al. (1983)
Mechanically	Protecon	Pork	Mixture	14.3		280			Bijker et al. (1983)
Mechanically	Protecon	Pork	Leg	15.6		110			Bijker et al. (1983)
Mechanically	Protecon	Pork	Shoulder	14.2		130			Bijker et al. (1983)
Mechanically	Protecon	Pork	Leg + shoulder	13.6		60			Bijker et al. (1983)
Mechanically	Protecon	Pork	Ribs and backs	15.6		120			Bijker et al. (1983)
Mechanically	Protecon	Pork	Shoulder	12.6		70			Bijker et al. (1983)
Mechanically	Protecon	Pork	Leg	14.7		70			Bijker et al. (1983)
Mechanically	Soeren	Pork	Leg + shoulder	12.5		90			Bijker et al. (1983)
Mechanically	Soeren	Pork	Ribs and backs	17.3		140			Bijker et al. (1983)
Hand-deboned	-	Poultry (chicken)	Breast	19.88	1.14	2.86	0.5	20.77	(Candoğan K et al., 2001)

Method	Mechanic method	Animal	Animal part	Protein %	Ash %	Calcium mg/100 g	Iron mg/100 g	Cholesterol mg/100 g	Reference
Hand-deboned	-	Poultry (chicken)	Neck	14.9	0.78	3.8	0.99	89.92	Candogan, et al., 2001
Hand-deboned	-	Poultry (chicken)	Back	17.31	0.65	3.09	1.27	98.13	Candogan, et al., 2001
Mechanically	-	Poultry (chicken)	Breast						Candogan, et al., 2001
Mechanically	-	Poultry (chicken)	Neck						Candogan, et al., 2001
Mechanically	-	Poultry (chicken)	Back						Candogan, et al., 2001
Mechanically		Poultry (turkey)						144	(King et al., 1998)
Hand-deboned		Poultry (chicken)	Breast					78.93	(Conchillo et al., 2005)
Hand-deboned		Pork	Tenderloin	21.1				62	(Buege et al., 1998)
Hand-deboned		Pork	Boneless sirloin chop	21.6				69	Buege et al. (1998)
Hand-deboned		Pork	Boneless loin chop	22.4				61	Buege et al. (1998)
Hand-deboned		Pork	Boneless rib roast	20.6				62	Buege et al. (1998)
Hand-deboned		Pork	Boneless loin roast	21				61	Buege et al. (1998)
Hand-deboned		Pork	Sirloin roast	20.4				66	Buege et al. (1998)
Hand-deboned		Pork	Loin chop	20.4				65	Buege et al. (1998)
Hand-deboned		Pork	Rib chop	19.2				66	Buege et al. (1998)
Hand-deboned		Pork	Ground	18				71	Buege et al. (1998)
Hand-deboned		Poultry (chicken)	Breast	20				77	Buege et al. (1998)
Hand-deboned		Poultry (chicken)	Drumstick	17.8				97	Buege et al. (1998)
Hand-deboned		Poultry (chicken)	Thigh	16.4				99	Buege et al. (1998)
Hand-deboned		Poultry (chicken)	Wings	16.6				107	Buege et al. (1998)
Hand-deboned		Poultry (chicken)	Ground	16.3				105	Buege et al. (1998)

B. ANALYSIS OF VARIANCE FOR CALCIUM CONTENT

Table 24: Analysis of variance for calcium content according to processing method, animal species and animal body parts.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Method	1	2450431	1835754	1835754	6.57	0.011
Species	1	376388	95318	95318	0.34	0.560
Part	5	437590	437590	87518	0.31	0.905
Error	185	51720037	51720037	279568		
Total	192	54984445				

GLOSSARY[15]

- **Fresh meat**: meat that has not undergone any preserving process other than chilling, freezing or quick-freezing, including meat that is vacuum-wrapped or wrapped in a controlled atmosphere.

- **Minced meat** means boned meat that has been minced into fragments and contains less than 1% salt.

- **Meat preparations** means fresh meat, including meat that has been reduced to fragments, which has had foodstuffs, seasonings or additives added to it or which has undergone processes insufficient to modify the internal muscle fibre structure of the meat and thus to eliminate the characteristics of fresh meat.

- **Mechanically separated meat or 'MSM'** means the product obtained by removing meat from flesh-bearing bones after boning or from poultry carcases, using mechanical means resulting in the loss or modification of the muscle fibre structure.

- **Low pressure MSM**: MSM produced using techniques that do not alter the structure of the bones used in the production of MSM and the calcium content of which is not significantly higher than that of minced meat.

- **High pressure MSM:** MSM produced using techniques other than those mentioned for low pressure MSM.

[15] According to Reg. (EC) 853/2004.